THE BOTANICAL GAZETTE

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS

THE CAMBRIDGE UNIVERSITY PRESS
LONDON

THE MARUZEN-KABUSHIKI-KAISHA
TOKYO, OSAKA, KYOTO, FUKUOKA, SENDAI

THE MISSION BOOK COMPANY
SHANGHAI

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THE

BOTANICAL GAZETTE

N. MANCHESTER, INDIA
PUBLIC

EDITOR
JOHN MERLE COULTER

VOLUME LXX

JULY–DECEMBER 1920

WITH FORTY-FOUR PLATES AND SIXTY-SIX FIGURES

B3.15

THE UNIVERSITY OF CHICAGO PRESS
CHICAGO, ILLINOIS

MISSOURI BOTANICAL
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Published
July, August, September, October, November, December, 1920

Composed and Printed By
The University of Chicago Press
Chicago, Illinois, U.S.A.

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DATES OF PUBLICATION

No. 1, July 24; No. 2, August 21; No. 3, September 15; No. 4, October 19; No. 5, November 24; No. 6, December 30.

ERRATA

VOL. LXX

P. 47, line 3, for "but as you suggested, it might be" read "but, as you suggested it might be,"

P. 211, line 13, for "LENA" read "LEVA"

P. 304, line 8 from bottom, for "change from wet to dry seasons" read "changes from wet to dry and dry to wet seasons respectively"

P. 333, line 4, for "*hyperforeum*" read "*hyberboreum.*

P. 359, in first plate title, for "*lyrata*" read "*lyratum*"

P. 524, line 16, for "centers" read "center"

P. 527, line 2, for "black, margined" read "black-margined"

VOLUME LXX NUMBER 1

THE
BOTANICAL GAZETTE

JULY 1920

DEVELOPMENT OF CYATHUS FASCICULARIS, C. STRIATUS, AND CRUCIBULUM VULGARE[1]

LEVA B. WALKER

(WITH PLATES I–VI AND THREE FIGURES)

A number of years ago when looking up literature on Gasteromycetes, the writer was impressed by the fact that no researches upon the development of any of the Nidulariaceae had been published since the classic papers of TULASNE (9), SACHS (8), EIDAM (4), BREFELD (1), and DEBARY (2, 3). From time to time, therefore, as suitable stages were found, the materials for these studies have been collected.

Cyathus fascicularis SCHW.[2]

Materials for the study of this form were collected largely in the various commercial greenhouses of Lincoln, Nebraska, where the fungus grows very abundantly during the late winter and early spring upon the wooden flats in which bulbs are planted for forcing. The mycelium is usually well developed upon the flats at the time they are taken from the storage cellars, and the basidiocarps usually mature at about the same time as the flowering of

[1] Contribution from the Department of Botany, University of Nebraska, New Series, no. 32.

[2] Material of this species was sent to Dr. E. A. BURT of the Missouri Botanical Garden for determination. He says: "It is certainly *C. fascicularis* Schw., which differs from the European specimens of *C. olla* Pers. in our herbarium in not having its sporangiales radiately rugose on the side attached to the funiculus. Although this difference is constant in all specimens examined, it is probably too slight for an adequate specific difference." (*C. olla* Pers. = *C. vernicosus* DC.)

the bulbs. By watching these flats, fruit bodies were found in all stages of development.

Materials for morphological studies were fixed in chrom-acetic (Flemming's or Benda's) solutions, dehydrated, cleared in either xylol or bergamot oil, and sectioned in paraffin. The most satisfactory results were obtained with Benda's solution. The basidiocarps were so filled with grit that many fruit bodies were treated with 10 per cent hydrofluoric acid for 48 hours before being dehydrated. The walls of the peridium and peridioles were at best very hard, especially in older basidiocarps, and this, together with the extreme gelatinization of the filaments which takes place during development, made the sectioning very difficult.

CULTURES.—Artificial cultures were first obtained during the early spring of 1914 from material collected at a local greenhouse. The inoculum used was the peridioles from the nearly mature but unopened basidiocarps. The fruit bodies were treated with a 1.5–1000 solution of mercuric chloride for a few minutes; the epiphragm was then removed with sterile tweezers, and the peridioles removed and planted on sterile agar media. Pure cultures were obtained repeatedly in this way, and also by searing the epiphragm with a hot scalpel and then removing the peridioles. An abundant pure white mycelium showing frequent clamp connections developed at once upon any of the ordinary agar media used in laboratories. Fig. 1 shows a culture of this kind which is five days old.

Mycelium in this condition was fixed, stained, and mounted in Venice turpentine. The mycelium branched abundantly, the branches turning at once in the direction of the growth of the main branch, and coming to lie near and parallel to the main branch. The mycelium was very constantly binucleate, and showed abundant clamp connections.

Mycelium from the agar cultures was transferred to sterile loam, old leaves, half rotten wood, etc., in flask cultures. On such media the mycelium made a very vigorous growth, and strong mycelial strands developed (figs. 2, 4–8). Cultures usually dried out before the formation of basidiocarps, and so water was added from time to time. In this way individual cultures were kept growing for several years. When cultures were kept in the light

a fresh crop of fruit bodies usually developed after each watering. No basidiocarps were found upon cultures kept in the dark, no matter how long the cultures were allowed to remain there. The best results were obtained by keeping the cultures in the dark for three or four months (or longer), until the culture medium seemed thoroughly permeated with mycelium, and then adding water just before placing them in the light, where fruit bodies developed in a few weeks. The most abundant development of basidiocarps

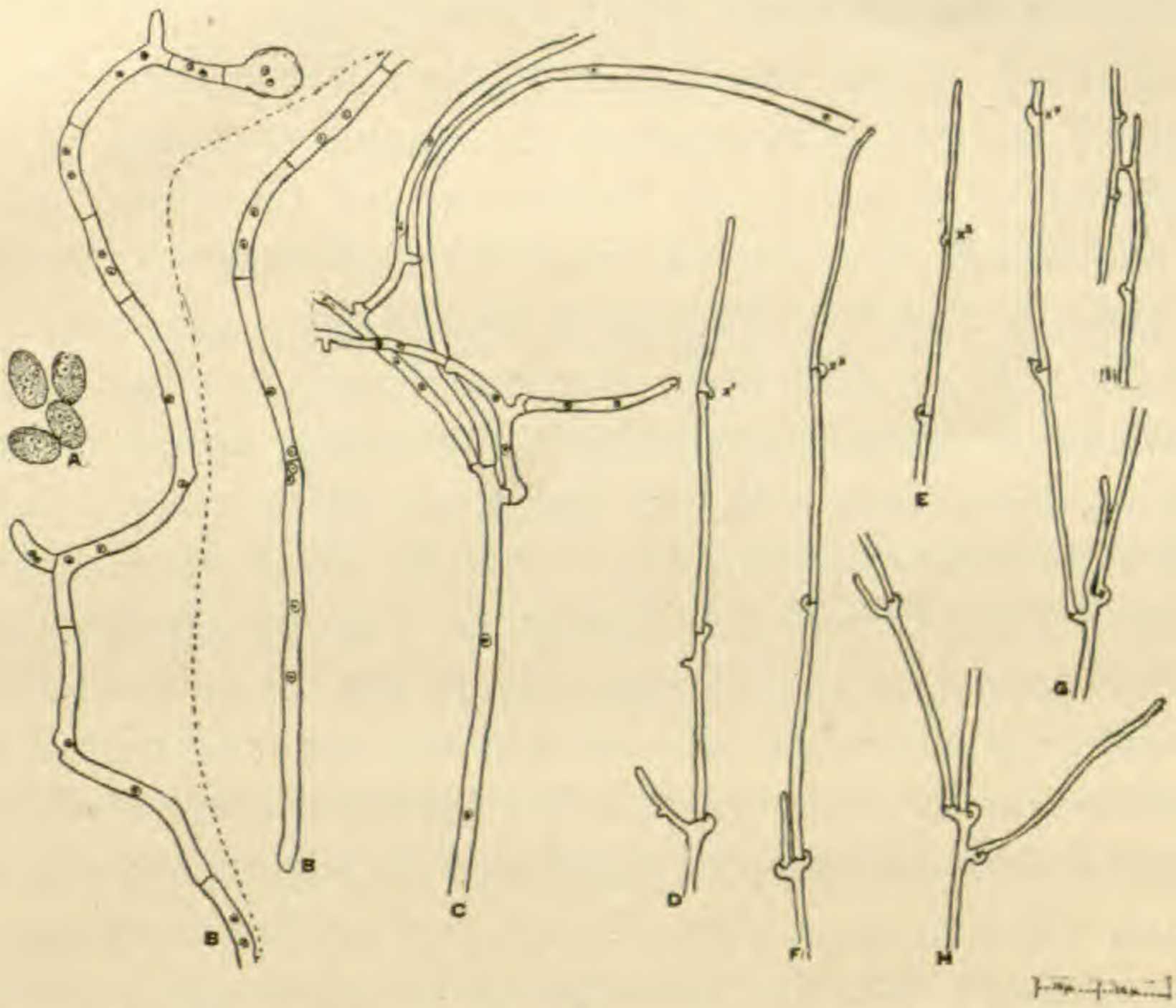

FIG. 1.—*A*, mature spores; *B*, germination spores; *C*, branching and nuclear conditions in older hypha; *D–H*, development of clamp connections (x^1–x^4) and relation of clamps to branching; *I*, anastomosing hyphae.

occurred in cultures exposed in an east window, where direct sunlight reached them during a few hours daily.

HANGING-DROP CULTURES.—Hanging-drop cultures of spores were made repeatedly, but usually spore germination did not take place for some reason. In a few cases, however, especially in one case in which spores from basidiocarps developed in pure cultures were used, abundant germinations in water were obtained. The germ tube usually came from the end of the spore and remained unbranched for some time. In a few cases branching very near

the spore or several germ tubes emerging from the spore were observed. The germinating spores were attached to the cover glass by allowing the water to evaporate until practically dry. They were then fixed in Flemming's fluid, dehydrated, and stained. The spores were constantly binucleate (text fig. 1*A*), and the mycelium developing from them was also composed of binucleate segments or segments which contained paired nuclei (text fig. 1*B*).

Small fragments of the tissue of the peridioles also developed mycelium in the cultures which grew much more extensively. Text fig. 1*C–I* shows the characteristics of the mycelium. In text fig. 1*C*, as well as in most of the mounts obtained where the protoplasmic structure could be determined, the mount was not dried sufficiently to make the mycelium adhere perfectly to the cover glass, so that the direction of growth does not show characteristically. Here, also, there is a binucleate mycelium provided with abundant clamp connections. The clamps appear quite constantly in connection with the branching of the main filaments as well as between the branches. Text fig. 1*D–I* shows in outline portions of a number of such filaments. Text fig. 1*D–G* illustrates the development of the clamps. Near the tip a branch turns backward just above the septum (x^1) and becomes united to the wall just below the septum (x^2). The intervening wall is absorbed (x^3), and a new wall is formed between the clamp and the apical cell from which it arose (x^4). So far as I could determine, there was no evidence that the formation of the clamps was associated constantly with nuclear divisions, as has been described by KNIEP (6) for certain Basidiomycetes. No mounts could be secured, however, in which both walls and nuclei showed distinctly enough in the same specimen to make satisfactory observations on this point. Hyphal anastomoses also occur occasionally (text fig. 1*I*).

MYCELIAL STRANDS.—As soon as growth in culture has reached a few centimeters, the filaments begin to show a tendency to coalesce to form mycelial strands, even upon agar media (fig. 3) and much more conspicuously upon loam, etc. (figs. 4–8). As development continues these strands enlarge until in some cases they become 0.5 mm. in diameter, as found in nature, but usually being 40–200 μ in diameter, the size seemingly being dependent upon the

available nutrition. Figs. 19 and 20 show transverse and longitudinal sections of strands. They are made up of many filaments lying almost parallel to each other and surrounded by a few loosely associated branches which form a vague outer sheath. The cells are very long and 2–5 μ in diameter, the larger being in the center.

ORIGIN OF BASIDIOCARPS.—The basidiocarps originate by the slight differentiation and enlargement of terminal portions of the mycelial strands (figs. 9, 10), and are first discernible in cultures as minute white knots on the mycelium (figs. 5, 6). The filaments of the strand branch in a fanlike manner at the base of the knot (fig. 10), and on the interior above become more slender (about 2 μ in diameter), much branched, and intricately interwoven, making a marked contrast with the filaments of the strand, which are larger and little branched. On the outer tip of the knot, however, is a tuft of hairs the same size as the cells of the mycelial strand. It seems evident, from a study of many slides, that the knot originates slightly below the tip of the mycelial strand, that these terminal hairs are the ends of the filaments which formerly made up the tip of the strand, and that the densely interwoven part just below represents the actual primordium of the basidiocarp. This closely interwoven part is very compact toward the lower part and more open toward the top, where large intermycelial spaces appear.

EARLY INTERNAL DIFFERENTIATION.—The first trace of internal differentiation is the gelatinization of a definite zone of tissue just below the part of the knot which shows the large intermycelial spaces. It extends downward toward the base of the knot in the form of an inverted dome (fig. 11). Fig. 24, between x–x, shows a higher magnification of this zone. The tissue just to the inside of this zone is made up of small, very densely interwoven filaments, and forms the line of demarcation between the primordium of the gleba and that of the peridium. The glebal primordium is composed of filaments of the same size and general character, but more loosely interwoven, so that large intermycelial spaces remain similar to those found in the earliest stages. Toward the top the filaments become more and more loosely interwoven, and pass

gradually into the large, long, flexuous hairs clothing the tip of the young basidiocarp. Fig. 14 shows a slightly older fruit body in which the gelatinization has progressed much farther, and in which the glebal portion shows much more numerous intermycelial spaces.

ORIGIN AND DEVELOPMENT OF PERIDIOLES.—The peridioles originate in the peripheral portions of the glebal region. They are distinguishable as spots where ends of filaments from all sides converge round a common point, as seen in fig. 12, and more highly magnified in fig. 25 in the region of intersection of lines from *a* to *a*. This mode of origin has also been described by FRIES (5) for *Nidularia*. The convergence first takes place in a semicircular manner, extending from the peridium inward toward the central part (fig. 25), but very soon the circle is completed around a common point of convergence. This region is surrounded by a zone of closely interwoven filaments such as originally made up the entire glebal region. A less dense zone with many intermycelial spaces soon tends to form a definite layer within the interlacing filaments, surrounding the converging filaments (except on the under side toward the peridium), and a slightly denser tissue is seen to the outside, which passes into the entirely undifferentiated ground tissue. These faintly marked zones represent the primordia of the walls of the peridiole, while the more or less parallel filaments which interrupt these zones on the under side, toward the peridium, represent the primordium of the funiculus.

The first peridiole to be differentiated is always near the base of the glebal tissue. Other peridioles follow successively from the base of the gleba upward, originating in the same manner from parts of the glebal primordium near its outer part. In the meantime the glebal region has elongated greatly (fig. 13), new growth taking place in an annular region near the apex of the fruit body, where the primordium of the gleba merges with that of the peridium. Fig. 16 shows a higher magnification of a part of the section seen in fig. 13. All the region just described can clearly be differentiated here. The densely interwoven filaments on the outside of the glebal region mark the inner border of the peridium, as will be described later.

As the development of the peridioles progresses, the circle of converging filaments enlarges, due to the multiplication of their elements, largely at the sides, and a central space is observed. This is well shown in figs. 26 and 28, which are higher magnifications of the peridioles in upper and lower portions of the basidiocarp seen in fig. 13. The central space is filled with slime, probably due to the gelatinization of filaments of the fundamental tissue, which are caught in the midst of the more actively growing converging filaments, as in older peridioles remnants of such filaments are clearly present (fig. 29). The cavity in the interior soon takes on an oval or bean shape (figs. 15, 17, 23). This is due to the earlier thickening of the walls of the peridioles on the upper and lower parts, while the sides (ends) remain thinner. Growth remains more active on the sides, and the addition of new elements here, together with mutual pressure, determines the final shape. The enlargement of the cavity in this way continues until the peridiole has reached its mature size.

The converging filaments lining the cavity are at first entirely undifferentiated, appearing in every way similar to the ends of actively growing vegetative filaments such as develop in culture media (figs. 26, 28). Soon they begin to enlarge on the end, and by the time the cavity has reached one-fourth to one-half its ultimate size a definite palisade layer, composed of filaments with enlarged ends, is formed (fig. 29). The palisade layer does not have a uniform even surface as in most of Basidiomycetes, but is made up of basidia and paraphyses of varying lengths. The cells forming the palisade layer are binucleate at first, but the nuclei soon fuse to form the primary nuclei of the basidia and the uninucleate paraphyses (figs. 27, 31). At maturity the entire cavity of the peridiole is densely filled with the oval, constantly binucleate spores (fig. 32).

For some time the walls of the peridioles remain only slightly differentiated (figs. 13, 16). In the subhymenium large intermyceliar spaces remain, and to the outside of this is a denser tissue which will develop into the thick inner wall of the peridiole. Soon, however, the ground tissue of the gleba begins to gelatinize in zones surrounding the already differentiated inner walls of the

peridioles. Figs. 15 and 18 show a basidiocarp where this gelatinization has only taken place around the lower peridioles, while figs. 17 and 21 show a slightly older stage. The tissue remaining between these gelatinizing regions and the denser filaments of the wall of the peridiole becomes the thin, colorless, outer wall of the peridiole. The filaments in this region gelatinize somewhat, but otherwise undergo little change. As the peridioles enlarge, this outer wall becomes stretched out (fig. 23) and remains as a colorless, delicate, easily removable coating over the surface of the mature peridiole. Text fig. 2*a* shows the structure of this layer.

FIG. 2.—Somewhat diagrammatic drawing of wall of mature peridiole through thinner region near side: *a*, outer wall; *b*, pseudoparenchymatic portion of inner wall; *c*, loosely interwoven portion of inner wall; *d*, hymenium.

The cells making up the extreme outer portion of the inner wall of the peridiole become much thickened, pseudoparenchymatic, and brown (fig. 23, text fig. 2*b*), and, showing through the outer hyaline wall, give the characteristic color to the mature peridiole. Within this layer of dark cells is a layer of compactly interwoven filaments whose walls are somewhat gelatinized. This gradually becomes looser as the hymenium is approached (fig. 27, text fig. 2*c*).

In some pure cultures basidiocarps arose between the culture medium and the glass (fig. 4). In such fruit bodies no peridium developed on the side next to the glass, and the whole development of the interior could be followed with a hand lens in an individual basidiocarp. Fig. 6 is an enlargement of the fruit body shown in fig. 4.

DEVELOPMENT OF FUNICULUS.—The funiculus has its origin in the somewhat parallel filaments extending from the innermost surface of the peridium to the primordium of the peridiole. This

appears as a darker area (fig. 25). Later, just to the outside of the inner wall of the peridiole, there appears, between the peridiole and the peridium, a region of actively growing filaments which take the stain readily (fig. 13; higher magnification of left hand peridiole, fig. 28). The filaments in this region elongate rapidly, and soon form a bundle of parallel filaments, as shown in fig. 30, which is a higher magnification of the lower left hand peridiole seen in figs. 15 and 18. Surrounding this bundle are the somewhat gelatinized filaments of the ground tissue, which will form the outer covering of the mature funiculus. The filaments which make up the central bundle of the funiculus continue very active growth, and, being confined by the gelatinizing filaments of the ground tissue, become coiled irregularly in this part. The filaments of the central cord always remain more or less parallel, even in the mature funiculus.

In the mature funiculus (fig. 23) the central cord is attached to the peridiole by the parallel filaments which marked its origin. It immediately twists to form the central coil of the funiculus, and from this passes abruptly at the base into a region of more delicate filaments which merge with the now gelatinizing tissue on the inner wall of the peridium. This central strand is surrounded by the gelatinizing filaments of the ground tissue which constitute its sheath.

The attachment of the funiculus to the peridium is always very weak in this species, so weak, in fact, that as one examines older fruits casually it is not evident, because the peridioles do not seem to be attached. An examination of the peridiole itself, however, shows that the well developed funiculus is always present.

DEVELOPMENT OF PERIDIUM AND EPIPHRAGM.—The mature peridium (fig. 33) is made up of three definite regions, a loosely interwoven outer layer composed of largely longitudinal filaments giving rise to hairs on the surface (*a*), a compact pseudoparenchymatic layer just within this (*b*), and a layer of loosely interwoven, more or less gelatinized filaments which extend from the pseudoparenchymatic layer to the glebal region (*c*).

In the development of the fruit body one can easily trace the origin of these layers. The differentiation takes place first at

the base, as can be observed in sections, and new growth takes place in an apical peripheral zone. This is also clearly shown in text fig. 3*A–D*, where two basidiocarps were marked with India ink, and the position of the lines observed on the older fruit bodies. The outer zone of the peridium comes from the loosely interwoven tissue on the outside of the young knots, which has undergone little differentiation from the condition in the mycelial strand (figs. 11, 12, 17), the zone of pseudoparenchyma, from the closely interwoven filaments within this; while the inner layer is made up of more or less gelatinized filaments which appear very differently at different stages in the development of the basidiocarp. The first differentiation seen in the fruit body is the beginning of gelatinization of the filaments of this inner zone, and in the younger basidiocarps it constitutes the great bulk of the tissue (figs. 13, 15, 17). As the fruit body matures, the continued enlargement of the glebal region causes this inner zone to become more and more compressed, until in a mature specimen it is seen as a zone no wider than the middle zone (fig. 23). At all times the inner zone shows three definite regions so distinctly that they might almost be considered as separate zones. Next to the middle pseudoparenchymatic zone the filaments are loosely interwoven, little if at all gelatinized, and lie longitudinally, following the outline of the middle zone. To the inside these give rise to the wide band of much gelatinized filaments which extend centripetally upward, and merge into the interwoven filaments which border the gleba.

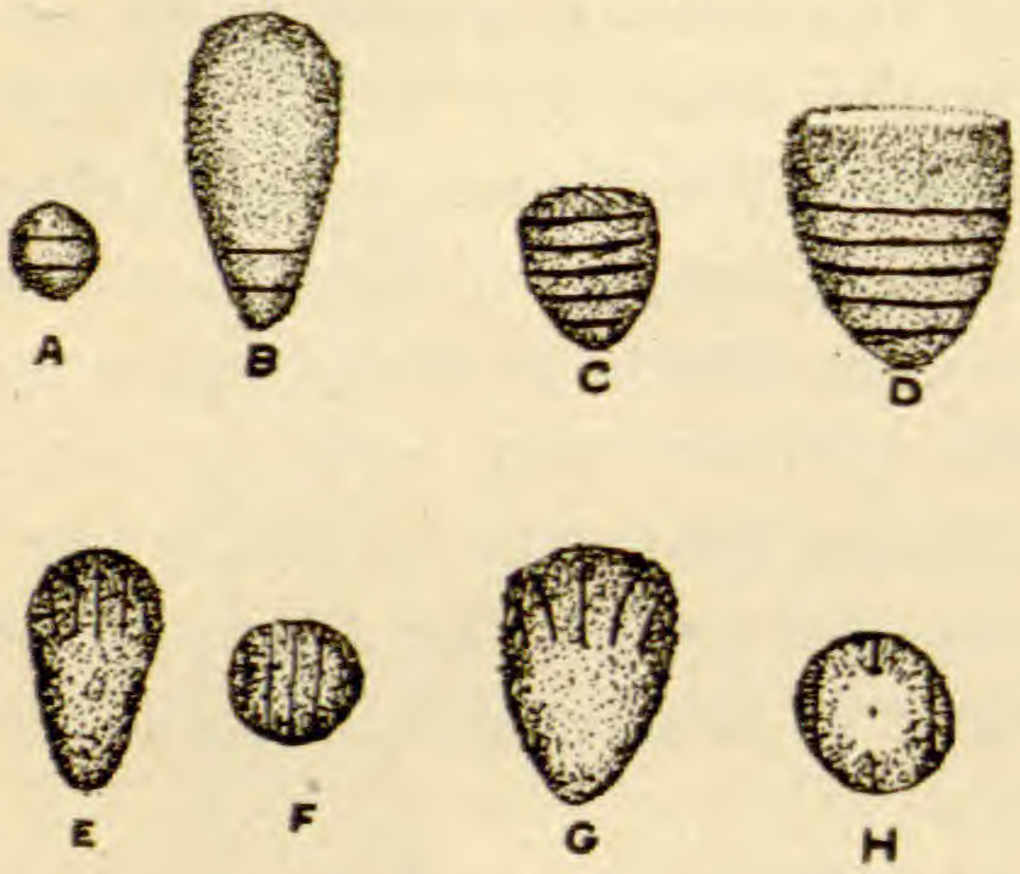

FIG. 3.—*A*, basidiocarp marked with India ink when 3 mm. high to determine region of growth; *B*, same 10 mm. high; *C*, basidiocarp marked when 5 mm. high; *D*, position of marks at time of expansion; *E*, *F*, side and end views of basidiocarp marked just before expansion; *G*, *H*, same basidiocarp with peridium breaking away and epiphragm showing in center.

The peridium covers all of the basidiocarp except the top. To the inside of the abrupt angle shown at the top of the fruit

body (figs. 17, 22) the middle or pseudoparenchymatic layer terminates, and the peridium over the top is composed of the filaments from the outer zone, which here give rise to especially long hairs, and of ungelatinized portions of the inner zone of the peridium.

As the fruit body matures lateral expansion takes place. This causes a rupture in this upper region, the parts structurally connected with the peridium being pulled off from over the top, leaving exposed the little differentiated ground tissue below (figs. 7, 8), which appears as a smooth uniform covering. Text fig. 3*E–H* illustrates how this takes place. A young basidiocarp of about the same age as the one shown in fig. 17 was marked with India ink as illustrated in *E* and *F*, while *G* and *H* show the condition a few days later.

Since the gelatinization of the ground tissue of the gleba begins at the base and progresses upward, the tissue at the top is the last to undergo gelatinization. This, together with the drying effect of the air, results in the formation of a thin membrane, the epiphragm, covering the top, which is exposed by the breaking away of the earlier covering parts. The epiphragm finally ruptures (fig. 5) and itself undergoes gelatinization, leaving the fruit body entirely open on the top. The moisture resulting from gelatinization dries out, and the peridioles sink to the bottom. Thus the basidiocarp assumes its characteristic mature appearance.

Cyathus striatus WILLD.

My studies of this species, based largely upon a small collection of only about 20 basidiocarps made during July 1913 on the campus of the University of Wisconsin at Madison, are necessarily quite incomplete. While this species is quite common in all regions where the writer has collected, it has been impossible to get young stages in most cases. It is included here because it displays some variations in development from that of *C. fascicularis*, and because of the close agreement with TULASNE's (9) work upon this species, and because TULASNE shows its close resemblance structurally to *C. fascicularis*.

The material was fixed in Flemming's medium and weak solutions, dehydrated, cleared in xylol, and sectioned in paraffin.

Cultures of *C. striatus* were made at various times, from material collected near Lincoln, Nebraska, by the same method as used for *C. fascicuiaris*. An abundant development of mycelium resulted, which was structurally much like that of *C. fascicularis*. The mycelium was at first white, but soon became a dirty brownish color, with many strong mycelial strands. In a few cases small knots appeared upon the mycelium, but mature basidiocarps were never developed.

YOUNG BASIDIOCARPS.—The youngest fruit bodies obtained were 2.5–4 mm. high, and the differentiation had advanced to a considerable degree, so that the peridial and glebal regions were well defined. Figs. 34 and 35 show very low and figs. 37 and 38 higher magnifications of fruit bodies of this type. They show about the same condition as is shown by TULASNE (**9**, *pl. 3, fig. 5*). The peridium has the same structure as in the mature basidiocarp (see fig. 48, the outside to the right), but it is not quite so much hardened. It is made up of three definite zones. The outer of these is made up of loosely interwoven, generally longitudinal filaments, which give rise on the outside to the dense covering of large, stiff, septate hairs about 6–10 μ in diameter; the middle is a pseudoparenchymatic layer which is much wider than the corresponding zone in *C. fascicularis;* while the broad inner layer is composed of more or less gelatinized filaments extending to the central top-shaped primordium of the gleba. This inner zone shows the three definite regions as described for *C. fascicularis*.

At the upper portion of the fruit body is an annular, deeper staining region where the peridial and glebal portions meet (figs. 37–40). This is the region of greatest growth, where new elements are added, both to peridium and gleba, during the elongation of the basidiocarp, just as in *C. fascicularis*. The top of the basidiocarp is covered densely with coarse hairs. The filaments from which these arise are much smaller and lie almost parallel to each other, thus forming a very definite zone over the upper portion of the gleba. The glebal region consists of closely interwoven filaments about 2 μ in diameter, resembling that of *C. fascicularis*.

ORIGIN AND DEVELOPMENT OF PERIDIOLES AND FUNICULUS.—The section shown in figs. 36 and 39 are of a basidiocarp 5 mm.

high when the peridioles are just appearing (cf. TULASNE 9, *pl. 3, fig. 6*). Their mode of origin is entirely like that of *C. fasciculare* as is shown in fig. 44, a higher magnification of the peridiole seen at the left near the base in fig. 39. The only variation in development is that the peridioles seem to appear simultaneously all through the glebal region. That they show in the photograph at the base is due to chance, for in examining the series it is clearly seen that they are equally distributed in all parts. As differentiation continues, we find the individual peridioles passing through identically the same stages as described for *C. fasciculare*, but while in that species the first peridiole differentiated was at the base and remained somewhat in advance of the others throughout its development, in *C. striatus* the upper peridioles soon outstrip the lower ones, so that the upper ones have formed a definite palisade layer before the lower ones have developed more than a small cavity in the center (figs. 40, 41). The same is true at the time of spore formation, the upper peridioles forming spores abundantly, while the lower ones still show a palisade condition. The gelatinization around the peridioles also takes place from above downward.

The structure of the mature peridiole differs from that of *C. fasciculare* only in its general proportions. All layers are much thinner, but especially is this true of the inner wall of the peridiole, which is often no more than 18 μ thick. Most of the apparent thickness of the wall in fig. 46 is due to the long stalks of the basidia which are shown in fig. 45. At maturity the interior of the peridiole is completely filled with the long, slender, binucleate spores characteristic of this species (fig. 47).

The funiculus also shows much the same course of development as in *C. fasciculare*. In the mature funiculus the filaments making up the coil become much more elongated, and therefore more twisted, and the strands forming the attachment to the peridial wall are more strongly developed. Figs. 41, 43, and 46 show stages in its development.

STRIATIONS ON WALL OF PERIDIUM.—The presence of the striations on the inner portion of the upper part of the peridium of the mature fruit body presents an additional point of interest. As the fruit body reaches maturity, the three layers of the peridial

wall extend practically across the top of the basidiocarp, quite in contrast with the condition in *C. fascicularis*. In this upper portion the peridium becomes folded, as seen in a somewhat tangential longitudinal section (fig. 42). At this time the peridioles reach practically to the top of the fruit body, as in younger stages (fig. 40). At the time of expansion this folded upper portion expands, forming the striate projection above the peridioles, which sink to the base of the fruit body as the gelatinized parts dry out.

Crucibulum vulgare TUL.

As *Crucibulum vulgare* is widely distributed and has been more extensively studied than other species of the Nidulariaceae, the writer was especially interested in obtaining it. The material used for most of my studies was discovered by Miss GERTRUDE E. DOUGLAS on an old gunny sack in the woods on the north shore of Beebe Lake on the Cornell University campus while we were collecting together in July, 1916. The old gunny sack was almost covered with fruit bodies in all stages of development. Knowing that I was especially interested in the group, she kindly turned the collection over to me for fixation and study. Material from this was fixed in Benda's solution, dehydrated, cleared in cedar oil, and imbedded in paraffin. Later more material was collected from an old board fence, also on the Cornell campus, and fixed in chrom-acetic solution. The fixation with this solution was not satisfactory, and little use was made of this collection except for purposes of comparison.

Artificial cultures were made of this fungus also, using the same methods as employed for *Cyathus fascicularis* and *C. striatus*. It made a vigorous growth upon all culture media tried. The mycelium was at first white, but soon became a dirty yellow and in parts quite brownish. Flask cultures, such as proved successful for *C. fascicularis*, were tried, but no mature fruit bodies were obtained. The mycelium showed much the same characteristics as that of *C. fascicularis*. Numerous knots appeared on the mycelial strands and on dense mats of mycelium from time to time, but never matured. MOLLIARD (7), however, obtained mature basidiocarps of *Crucibulum vulgare* in similar pure cultures after two and

a half years. The writer's cultures were never maintained for so long a period.

ORIGIN OF BASIDIOCARP.—The basidiocarps arose from mycelial strands or from densely interwoven vegetative mats of hyphae. Not uncommonly young fruit bodies developed on the inside of old peridia which had lost their peridioles. The youngest basidiocarps obtained were about 0.5 mm. in height and showed no internal differentiation, except that the mass took the stain slightly more deeply at the top where the filaments were much smaller (about 1.5 μ in diameter) and more closely interwoven than at the base. Figs. 49–52 show some of these young basidiocarps, while fig. 65 is a higher magnification of the upper portion of the fruit body shown in fig. 50. At the base of the knot the filaments are larger (about 2.5–3 μ in diameter), where they spring from a mycelial strand or mat, and branch at first in a fan-like manner, soon becoming much branched and interwoven as they ascend, and gradually passing into the smaller filaments at the top. In the region made up of these small, closely interwoven filaments many large intermycelial spaces are present (fig. 65). Even these youngest fruit bodies are covered densely with yellow, much branched, thick-walled hairs with toothlike projections (fig. 65). These hairs are 3.5–4 μ in diameter, and so conspicuous that the smallest basidiocarps found could be distinguished only by the presence of yellow tufts made up of these hairs.

INTERNAL DIFFERENTIATION OF BASIDIOCARPS.—The first differentiation here, as in *Cyathus fascicularis*, consists in the gelatinization of filaments. This zone is very vague in its early stages, and it is difficult to be certain just when it begins. Filaments just beginning to gelatinize seem to take the stain more deeply than ungelatinizing filaments, and soon to lose this power and take the stain less deeply. Using these properties and a very slight difference in appearance as determining factors, it seems that the gelatinization begins at the base of the fruit body (fig. 52) and progresses upward in an annular fashion (fig. 53), the darker regions on the sides representing the region just beginning to gelatinize, and the lighter region below representing the more gelatinized filaments. SACHS (8), in his description of the development of

the basidiocarps of *Crucibulum*, also finds that the gelatinization begins at the base and takes place upward in the same manner.

This zone of gelatinization soon becomes a well marked one (fig. 55), extending from the upper peripheral part of the fruit body downward, thus outlining the tissue to the interior, the primordium of the gleba, which has undergone no further differentiation. As the filaments to the outside of the glebal region become more and more gelatinized, they elongate and extend in a downward and outward direction to the outside of the deeply stained filaments bordering the glebal primordium (fig. 55). A pronounced lateral expansion of the basidiocarp results. Fig. 66, a higher magnification of the upper left hand portion of fig. 55, shows the structure of the glebal primordium and the tissues bordering it. This is the condition of the fruit body just before peridiole formation.

Fig. 56 shows a fruit body slightly older than the one shown in fig. 55, with the peridioles just beginning to appear. As in *C. fasciculariis* and *C. striatus*, the first trace of peridiole formation is found in the appearance of regions near the outer part of the glebal region, where the filaments point radially toward a common center. Fig. 67 shows a higher magnification of the peridiole primordium seen at the base of the fruit body in fig. 56. These inward pointing filaments soon become surrounded by a layer of densely interwoven filaments, and this layer by a region with many intermycelial spaces, which is only slightly interrupted on the side toward the peridium by filaments which lie more parallel to each other and extend downward (fig. 67) to a deeper staining region just outside the zone with intermycelial spaces. This is the primordium of the funiculus.

The further development of the peridiole takes place much as in *C. fasciculariis*, and even in its final differentiation shows only variations in minor details (figs. 56–59, 61–62, 70). The peridioles here, contrary to the observations of SACHS, seem to appear in all parts of the gleba almost simultaneously, as in *C. striatus*, and seem to develop almost uniformly in all parts of the fruit body. The only variation was that the lowest peridiole is quite uniformly smaller and sometimes not so well developed. Fig. 64

shows the interior of a mature peridiole with the cavity almost completely filled with the oval binucleate spores. In thickness and consistency the walls of the peridiole are more like those of *C. fasciculare*, and like it are much thinner on the ends than on the sides.

In *Crucibulum*, however, the filaments of the outer wall remain entirely ungelatinized for a longer time, and this tissue is more definitely separated from the gelatinizing filaments surrounding it by a thin border layer of filaments which soon become entirely gelatinized. The remnants of these gelatinized filaments bordering the peridioles take the red stains (saffranin and fuchsin) deeply. thus giving the very conspicuous border to the outer wall of the peridioles as seen in fig. 70. As can be seen in figs. 58 and 61, much more of the undifferentiated glebal tissue remains and undergoes complete gelatinization than in the species of *Cyathus* studied.

The funiculus has its origin, as already pointed out, in a region of more active growth just to the under side of the young peridiole (figs. 56, 67). In this region the filaments elongate very rapidly, and soon develop into a stout bundle of parallel filaments extending from the young peridiole to the glebal wall (figs. 57, 68). The filaments forming this bundle are surrounded by gelatinizing filaments which extend in the same general direction, and will form the sheath of the funiculus. The central strand of parallel filaments continues its elongation as the basidiocarp develops, and by the time a palisade layer is differentiated (figs. 58, 59) two definite regions are easily distinguishable, a region of much coiled and twisted filaments forming a knot just below the peridiole, and a long slender strand reaching down much below the present position of the peridiole and widening out as it attaches itself to the peridium (fig. 59). This was also described by TULASNE.

These strands are easily traceable far down the sides of the peridium by the conspicuous clamp connections which are abundantly present (fig. 60). The position of the clamps indicates that growth in the strand is upward from the peridial region. Among the gelatinizing filaments surrounding the central strand of the funiculus can clearly be seen the less gelatinized filaments making up the sheath of the funiculus (figs. 61, 62, 70). A funiculus from a nearly mature basidiocarp is shown in figs. 69 and 70.

DEVELOPMENT OF PERIDIUM AND EPIPHRAGM.—The mature peridium (fig. 63) would closely resemble that of *Cyathus fascicularis* and *C. striatus* if the middle pseudoparenchymatic layer were omitted. The outer layer consists of loosely interwoven, largely longitudinal filaments giving rise to long flexuous hairs on the outside. Among these hairs can be seen only rarely, more often toward the top, traces of the much branched, sharp pointed hairs that originally covered the entire fruit body. The filaments comprising the outer part are thick-walled and brownish, becoming thinner-walled and colorless toward the inner portion, and thus merging into the inner layer, which is composed of more or less gelatinized filaments. This peridium covers the sides and converges slightly over the top of unopened fruit bodies (fig. 61).

These zones of the peridium are quite well differentiated before the primordia of the peridioles appear, and the outer zone undergoes little change during subsequent development. On the other hand, the inner zone changes greatly. In the young fruit bodies (figs. 55, 56) it is a wide zone, but as the glebal region develops it becomes more and more compressed (figs. 57, 59), until in the mature basidiocarp it forms an almost indistinguishable layer (figs. 61, 70).

The epiphragm covers the upper surface of the fruit body and is definitely marked by the time the basidiocarp is half developed (fig. 58). It consists of the entirely undifferentiated upper part of the basidiocarp as seen in younger stages (figs. 55, 56), and is as densely covered by the much branched, pointed hairs as was the original young fruit body (figs. 49–54). Following the gelatinization of the filaments surrounding the peridioles, those constituting the epiphragm also undergo gelatinization. The epiphragm just before gelatinization is shown in fig. 62. There is no opening up of the upper portion by the spreading of the superficial layer as described for *C. fascicularis*.

Comparisons

From FRIES's description of the development of *Nidularia* it evidently has the simplest structure of any of the Nidulariaceae whose development has been studied. Its development seems to correspond very closely with that of *Crucibulum*, except that the

funiculus is entirely lacking. Both are covered at first with toothed hairs, and the internal structures are similar. The zone of gelatinization, which is the first differentiation to be observed, is similar, except that in *Nidularia* it seems to extend over the top of the basidiocarp also. The development in both seems to be basal, as shown by the location of the toothed hairs on the upper portions of mature basidiocarps. The tissues covering the top of the fruit body just before opening are seemingly homologous, but more definitely limited in *Crucibulum*. The structure of the peridium and the origin and development of the peridioles are similar, but in *Nidularia* they appear first at the base, while in *Crucibulum* they appear simultaneously. The structure of the walls of the peridioles, as described by FRIES, is very different seemingly. The close relationship of these forms, however, is very evident.

In *Cyathus* more differences appear, especially in the structure of the peridium and funiculus, but the general type of development is very similar. Of the forms studied, *C. fascicularis* shows closer resemblances to *Crucibulum* than does *C. striatus*. The part of the gleba to become differentiated first and mature first seems to be very variable in the genera and species studied. While in my materials it has seemed to remain quite constant for each species, there is the possibility that it may vary even in the same species.

Summary

1. All three species are easily grown on artificial media. Mature fruit bodies were obtained only in cultures of *Cyathus fascicularis*.

2. The mycelia of all are very similar except for color. Clamp connections are abundantly present, and conspicuous mycelial strands are formed. The cells of *Cyathus fascicularis* are binucleate or composed of segments with paired nuclei.

3. The basidiocarps of *Cyathus fascicularis* and *C. striatus* arise from mycelial strands in all cases observed; while those of *Crucibulum vulgare* may arise from mycelial strands, dense mats of hyphae, or from the interior of old peridia.

4. The primordium of the basidiocarp seems to have its origin slightly below the tip of the strand, and consists of closely interwoven filaments smaller than those of the strand.

5. The first marked internal differentiation in all three consists of the gelatinization of a zone of hyphae in a region that will become a part of the inner wall of the peridium. A zone of closely interwoven filaments just to the inside of this forms the boundary between gleba and peridium.

6. The origin and development of the peridioles is similar in all. Each peridiole originates around a center, toward which the ends of filaments converge. The structure of the walls of the peridioles differs only in relative proportions.

7. The first peridioles to be differentiated in *Cyathus fasciculatis* are toward the base of the gleba, and later other peridioles develop above them. The peridioles at the base mature before those nearer the top of the gleba. In *C. striatus* and *Crucibulum vulgare* the peridioles appear almost simultaneously throughout the glebal region; but the upper peridioles in *C. striatus* mature before the lower ones; while in *C. vulgare* the development is more uniform.

8. The funiculus of *Crucibulum* differs greatly in form from that of *Cyathus*, especially in the length of the strand at the base. The origin is similar in both.

9. The most marked difference between *Crucibulum* and *Cyathus* is in the structure of the walls of the peridia. In *Cyathus* a middle pseudoparenchymatic layer is present which is entirely wanting in *Crucibulum*.

10. During the expansion of the basidiocarp in *Cyathus* the peridium is pulled off from over the glebal region, leaving parts of the ungelatinized ground tissue to form, for a time, a thin covering (the epiphragm). In *Crucibulum* the epiphragm consists of the undifferentiated primordial tissue covered with branched hairs. This undergoes gelatinization at maturity.

11. The spores of all are constantly binucleate.

I wish to express my indebtedness to the late Professor GEORGE F. ATKINSON, who saw a first draft of this paper, for a number of helpful suggestions, to Miss GERTRUDE E. DOUGLAS for the collection of much of the material used in the study of *Crucibulum vulgare*, and to Dr. E. A. BURT for going over the manuscript of

this paper with me and making suggestions, and for the determination of *Cyathus fascicularis*.

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EXPLANATION OF PLATES I–VI

Figs. 1–8 were made with a Century camera and Zeiss-Tessar lens. The photomicrographs were made as follows: figs. 9–23, 34–43, 46, 49–59, 61, 62, with a horizontal Zeiss camera; figs. 19, 20, 24–33, 44, 45, 47, 48, 60, 63, 64, 69, 70, with a Bausch and Lomb vertical camera and Zeiss lenses; and figs. 65–68 with a Bausch and Lomb vertical camera and Leitz lenses.

PLATES I–III

Cyathus fascicularis

FIG. 1.—Culture growing from peridiole, 5 days old; $\times 1\frac{3}{4}$.
FIG. 2.—Young culture growing on wood and loam; $\times \frac{1}{2}$.
FIG. 3.—Tube culture showing beginnings of strand formation; $\times \frac{7}{8}$.
FIG. 4.—Basidiocarps developing between loam and sides of culture jar in which no peridium formed on side next to glass; $\times \frac{1}{2}$.
FIG. 6.—Basidiocarps shown in fig. 4; $\times 2$.

FIGS. 5, 7, 8.—Basidiocarps and mycelial strands formed in cultures, slightly reduced in size.

FIG. 9.—Median longitudinal section of young basidiocarp with mycelial strand from which it developed; ×31.

FIG. 10.—Slightly older basidiocarp, cut somewhat diagonally to show relation of filaments of strand to basidiocarp; ×31.

FIG. 11.—Median longitudinal section of basidiocarp showing origin of inverted gelatinous dome, which is first internal differentiation to take place; ×31.

FIG. 12.—Median longitudinal section of basidiocarp with zone of gelatinization well defined and origin of peridioles evident; ×31.

FIG. 13.—Longitudinal section (median at top, little to one side of center at base) of basidiocarp, with all parts of mature fruit body distinguishable; ×13.

FIG. 14.—Median longitudinal section of basidiocarp intermediate between figs. 11 and 12; ×31.

FIG. 15.—Median longitudinal section of basidiocarp, somewhat older than fig. 13; ×13.

FIG. 16.—Higher magnification of portion of basidiocarp shown in fig. 13; ×31.

FIG. 17.—Median longitudinal section of basidiocarp slightly older than fig. 15; ×13.

FIG. 18.—Higher magnification of portion of section shown in fig. 15; ×31.

FIG. 19.—Transection of mycelial strand; ×225.

FIG. 20.—Longitudinal section of mycelial strand; ×225.

FIG. 21.—Higher magnification of lower peridiole shown in fig. 17; ×31.

FIG. 22.—Higher magnification of upper portion of section shown in fig. 17; ×31.

FIG. 23.—One peridiole, with funiculus and portion of peridium from longitudinal section of basidiocarp; ×31.

FIG. 24.—Higher magnification of gelatinizing zone (*x*–*x*) from section shown in fig. 11; ×225.

FIG. 25.—Higher magnification of earliest trace of peridiole differentiation; center of converging filaments at point of intersection of line *A*–*A*; ×225.

FIG. 26.—Higher magnification of peridiole from upper portion of fruit body shown in figs. 13 and 16; ×225.

FIG. 27.—Hymenium and subhymenium as seen in peridiole with mature spores; ×225.

FIG. 28.—Higher magnification of peridiole shown at lower left hand side of figs. 13 and 16; ×225.

FIG. 29.—Section of peridiole about one-half mature size, showing structure of hymenium and tissues below it; ×225.

FIG. 30.—Funiculus of peridiole shown in fig. 29; ×225.

FIG. 31.—Much higher magnification of small portion of hymenium from peridiole just beginning to form spores, showing young binucleate basidia and uninucleate basidia; ×585.

FIG. 32.—Mature binucleate spores from same peridiole as fig. 31; ×585.

FIG. 33.—Longitudinal section of peridium of mature basidiocarp: *a*, loose outer layer; *b*, pseudoparenchymatic layer; *c*, inner, loosely interwoven, and more or less gelatinized layer; ×225.

PLATE IV

Cyathus striatus

FIGS. 34, 35.—Median longitudinal sections of youngest basidiocarps found (basidiocarp in fig. 35 bent at base so that section of lateral wall is seen at base); ×14.

FIG. 36.—Median longitudinal section of older basidiocarp with peridioles appearing; ×14.

FIGS. 37–39.—Higher magnifications of basidiocarps shown in figs 34, 35, 36; ×34.

FIG. 40.—Median longitudinal section of still older basidiocarp; ×73.

FIG. 41.—Higher magnification of portion of section shown in fig. 41; ×34.

FIG. 42.—Tangential longitudinal section showing folding of incurved portion of peridium; ×31.

FIG. 43.—Section of nearly mature peridiole; ×34.

FIG. 44.—Higher magnification of portion of section seen in fig. 39 showing converging filaments in early development of peridiole; ×225.

FIG. 45.—Section of peridiole producing spores, showing hymenial layer; ×225.

Fig. 46.—Section of peridiole producing spores and funiculus; ×225.

FIG. 47.—Mature spores; ×360.

FIG. 48.—Section of peridium of mature basidiocarp; ×225.

PLATES V, VI

Crucibulum vulgare

FIGS. 49–52.—Median longitudinal sections of very young basidiocarps; ×31.

FIGS. 53–55.—Median longitudinal sections of basidiocarp showing early stages in gelatinization of filaments bordering primordium of gleba; ×31.

FIG. 56.—Median longitudinal section showing origin of peridioles; ×31.

FIG. 57.—Slightly tangential longitudinal section showing early stage in development of peridiole and funiculus; ×31.

FIG. 58.—Longitudinal section of older basidiocarp; ×13.

FIG. 59.—Section of peridiole before spore formation, and funiculus; ×31.

FIG. 60.—Section of mature funiculus showing clamp connections; ×360.

FIG. 61.—Longitudinal section of nearly mature basidiocarp; ×13.

FIG. 62.—Portion of longitudinal section of older basidiocarp; ×31.

FIG. 63.—Longitudinal section of peridium of mature basidiocarp; ×225.

FIG. 64.—Section of peridiole showing hymenium and interior filled with spores; ×360.

FIG. 65.—Higher magnification of upper portion of basidiocarp shown in fig. 50; ×225.

FIG. 66.—Higher magnification of upper left hand portion of fig. 55; ×225.

FIG. 67.—Higher magnification of peridiole initial near base of basidiocarp shown in fig. 56; ×225.

FIG. 68.—Higher magnification of funiculus to peridiole shown to left in fig. 57; ×225.

FIG. 69.—Higher magnification of upper portion of funiculus shown in fig. 70; ×360.

FIG. 70.—Funiculus from another section of basidiocarp shown in fig. 61; ×31.

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WALKER on CYATHUS

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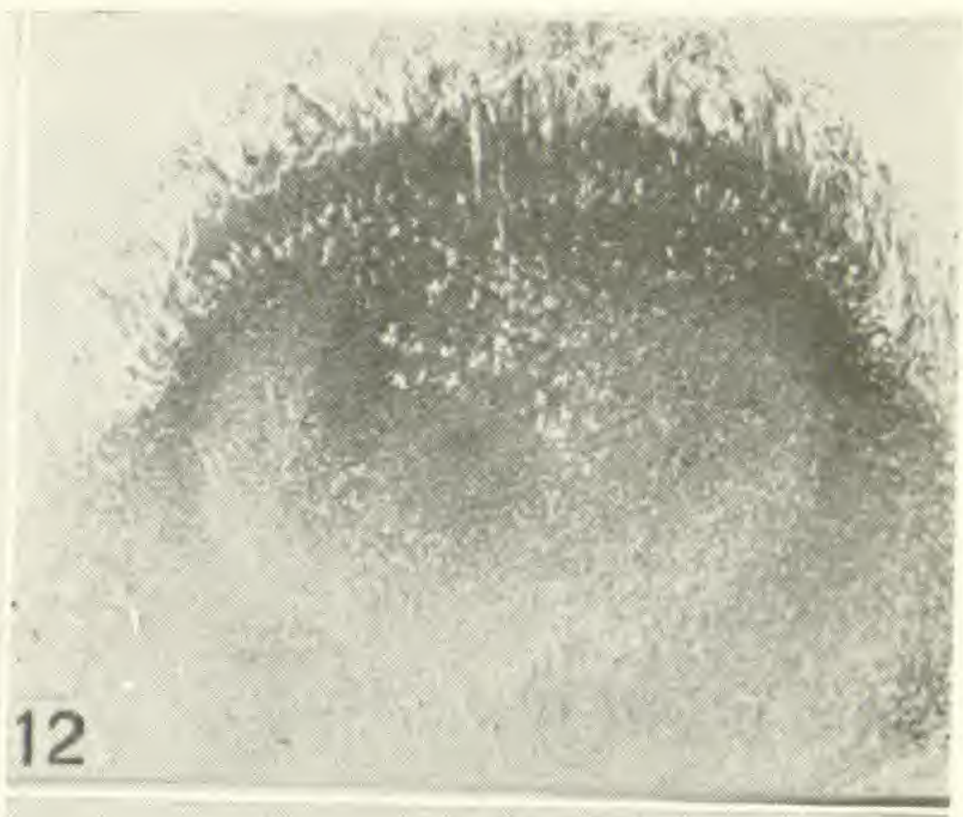
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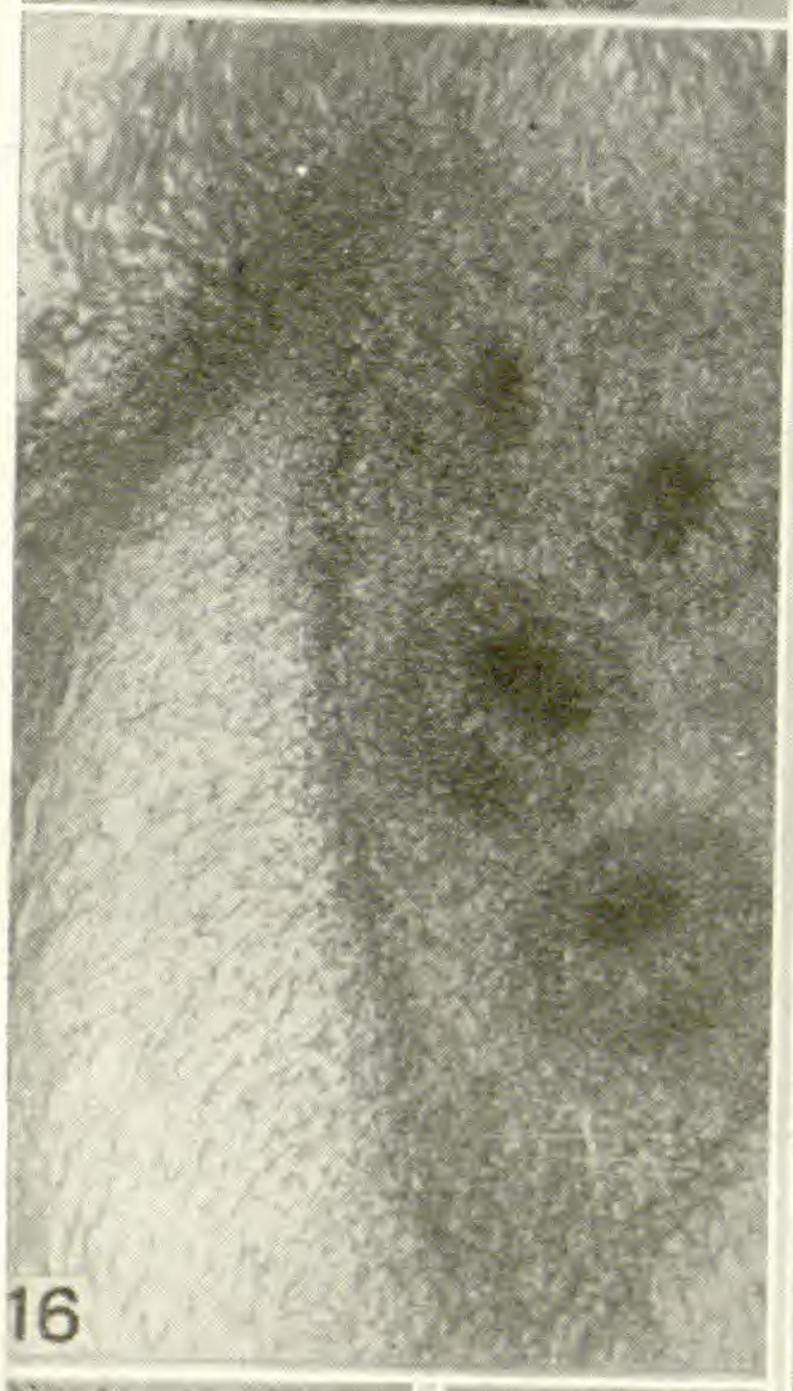
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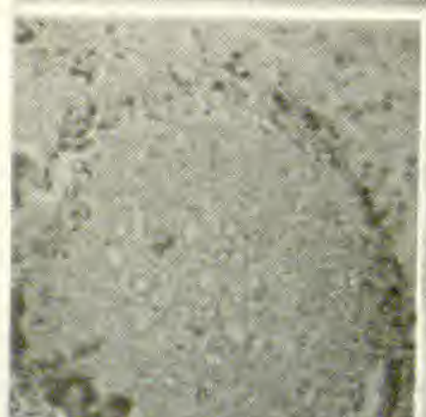
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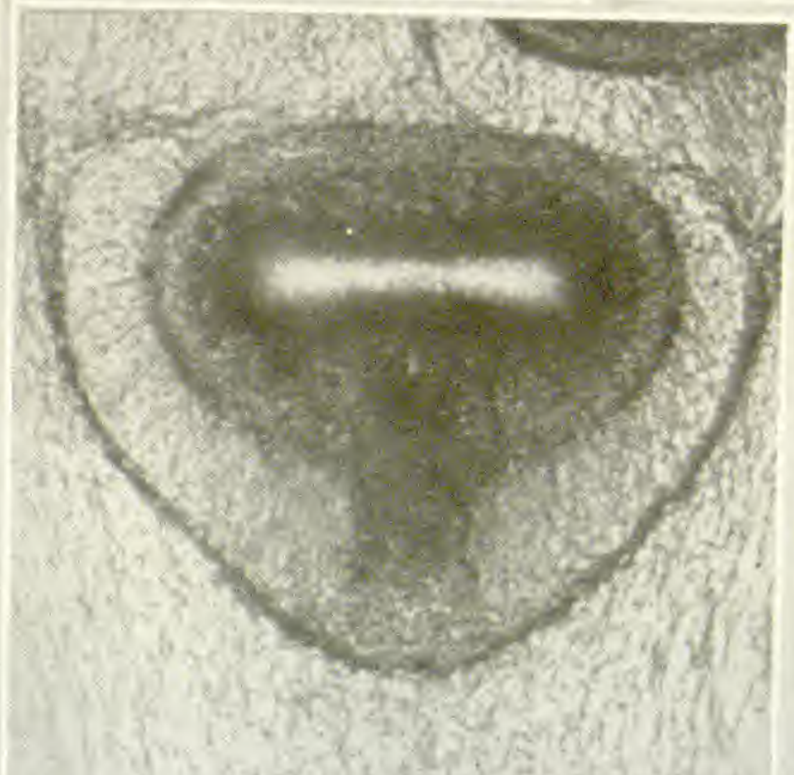
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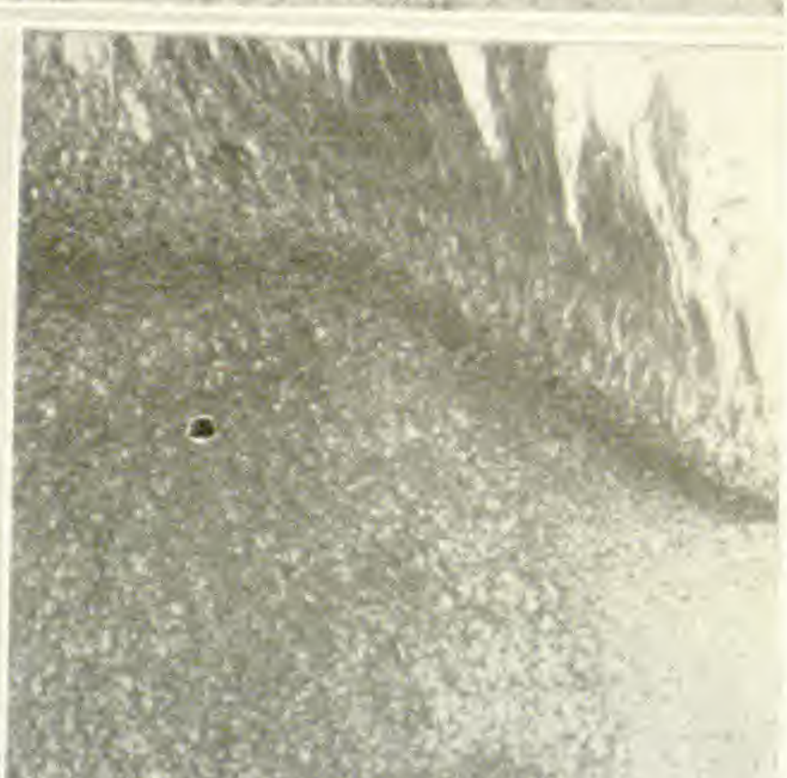
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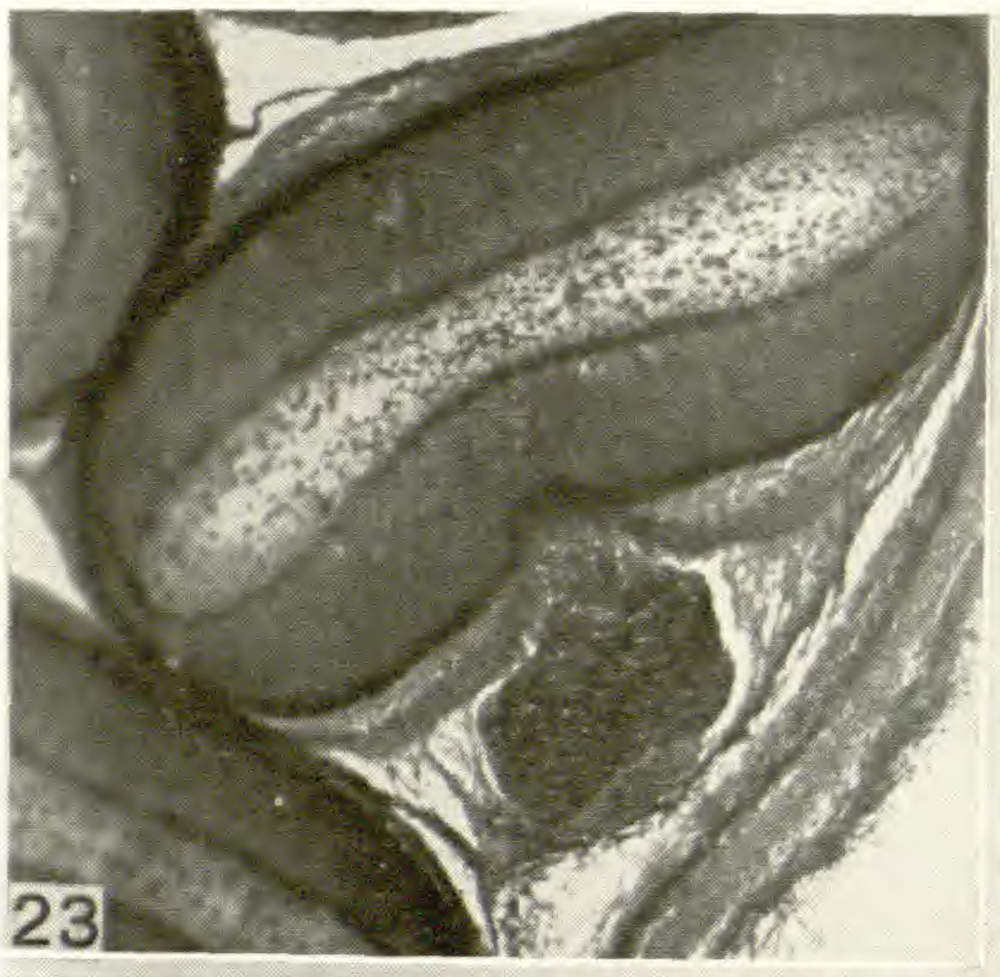
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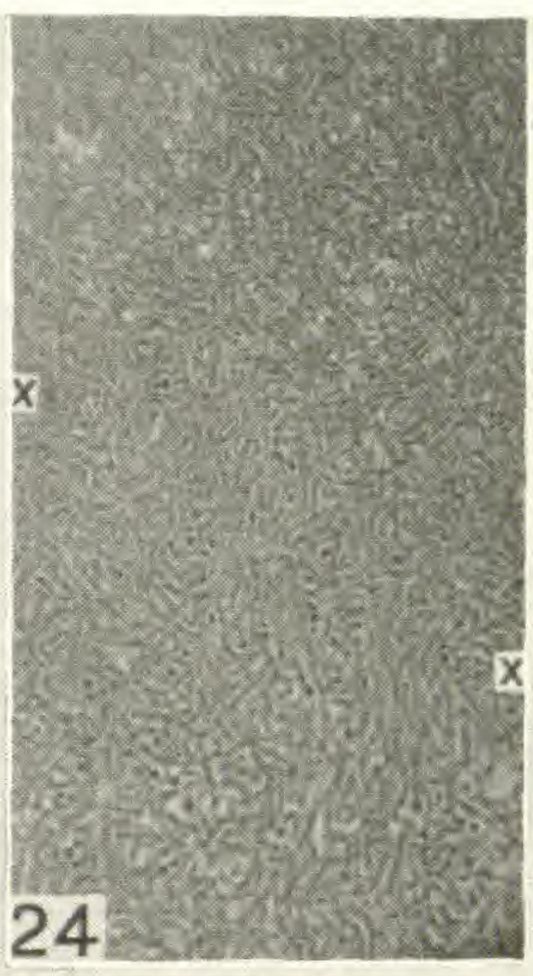

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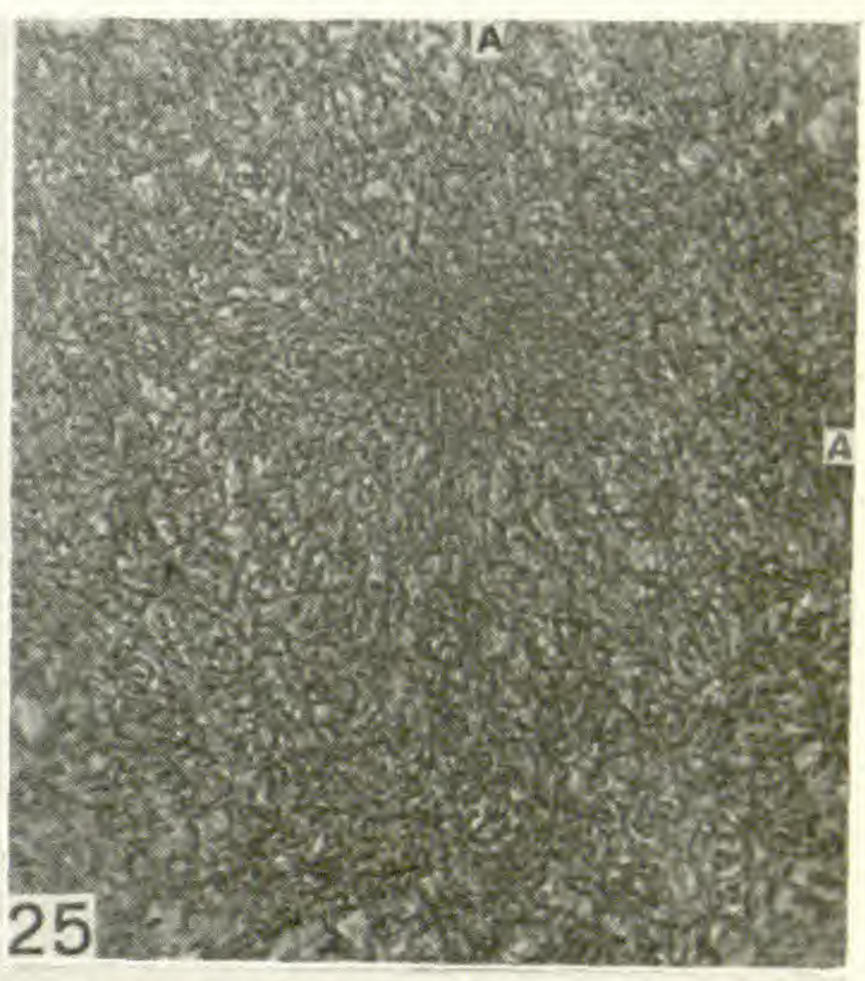

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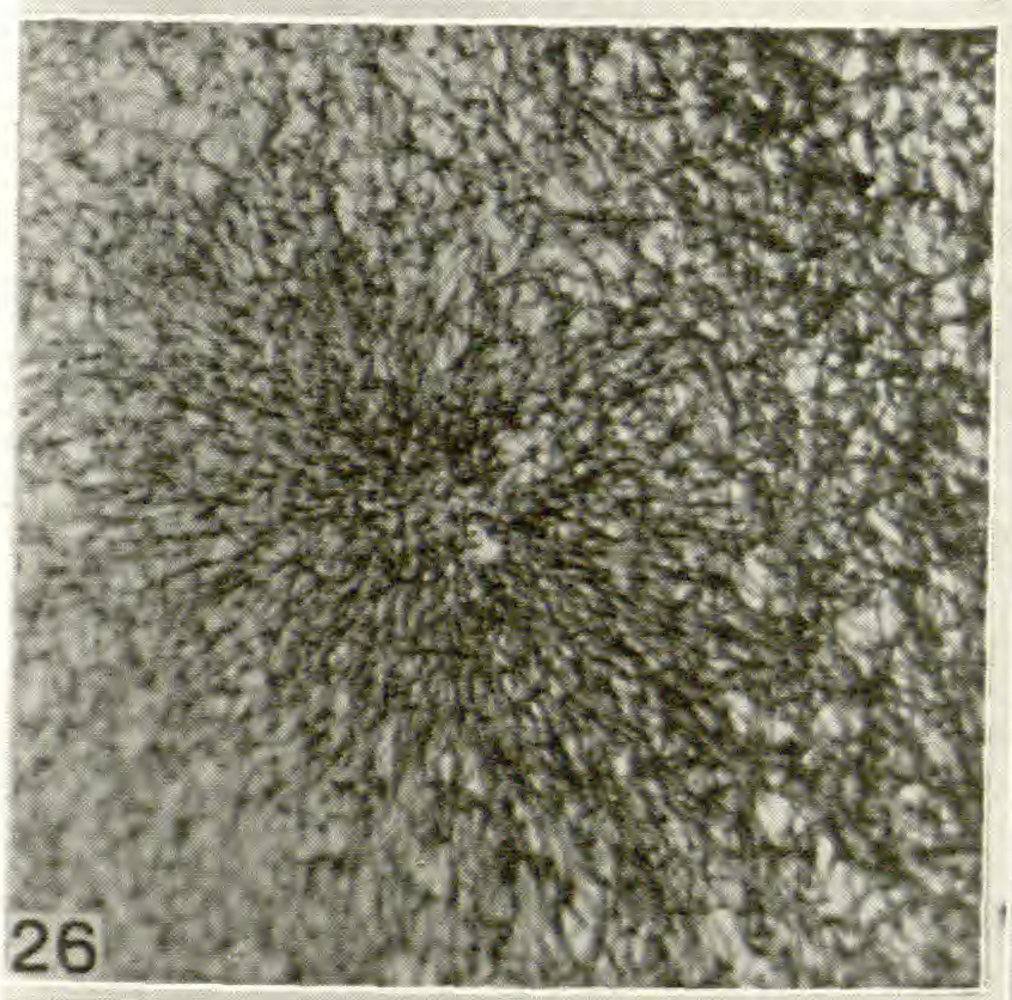
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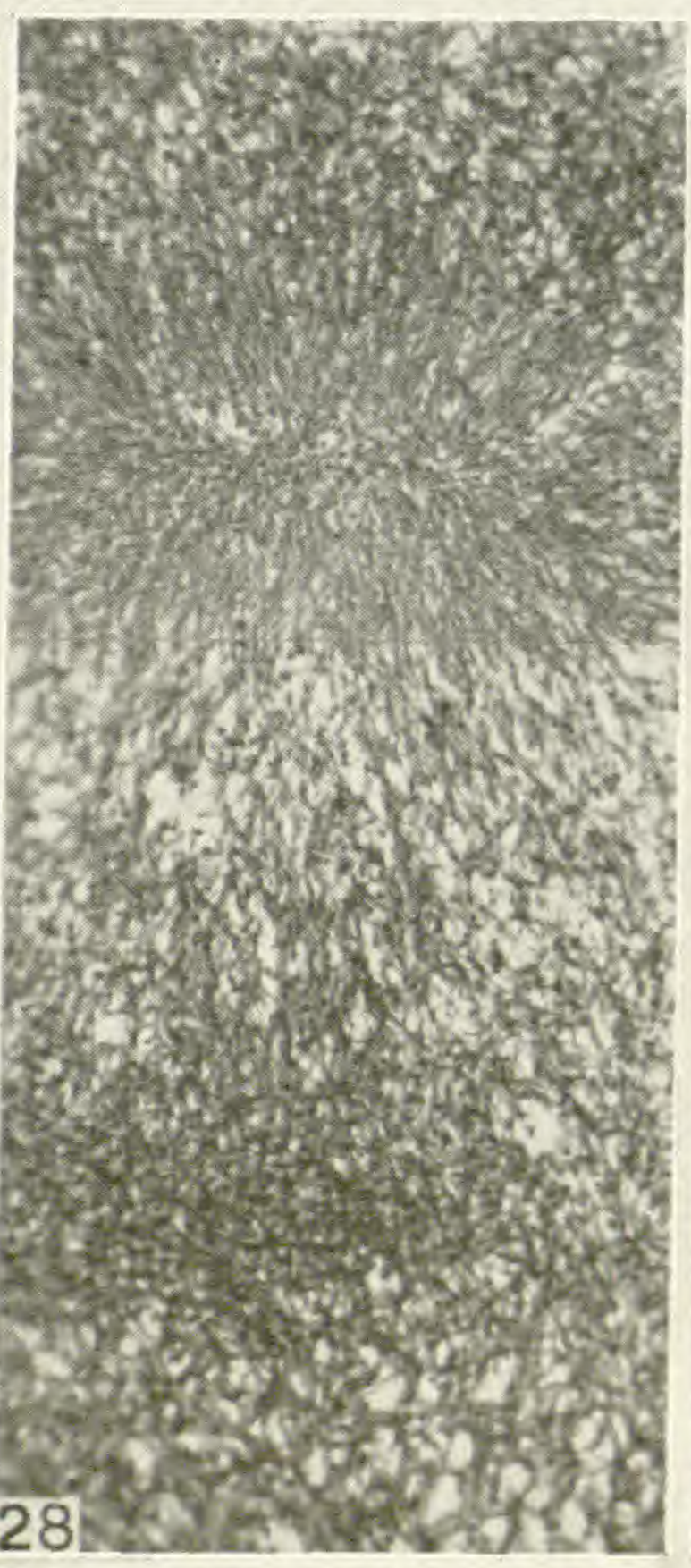
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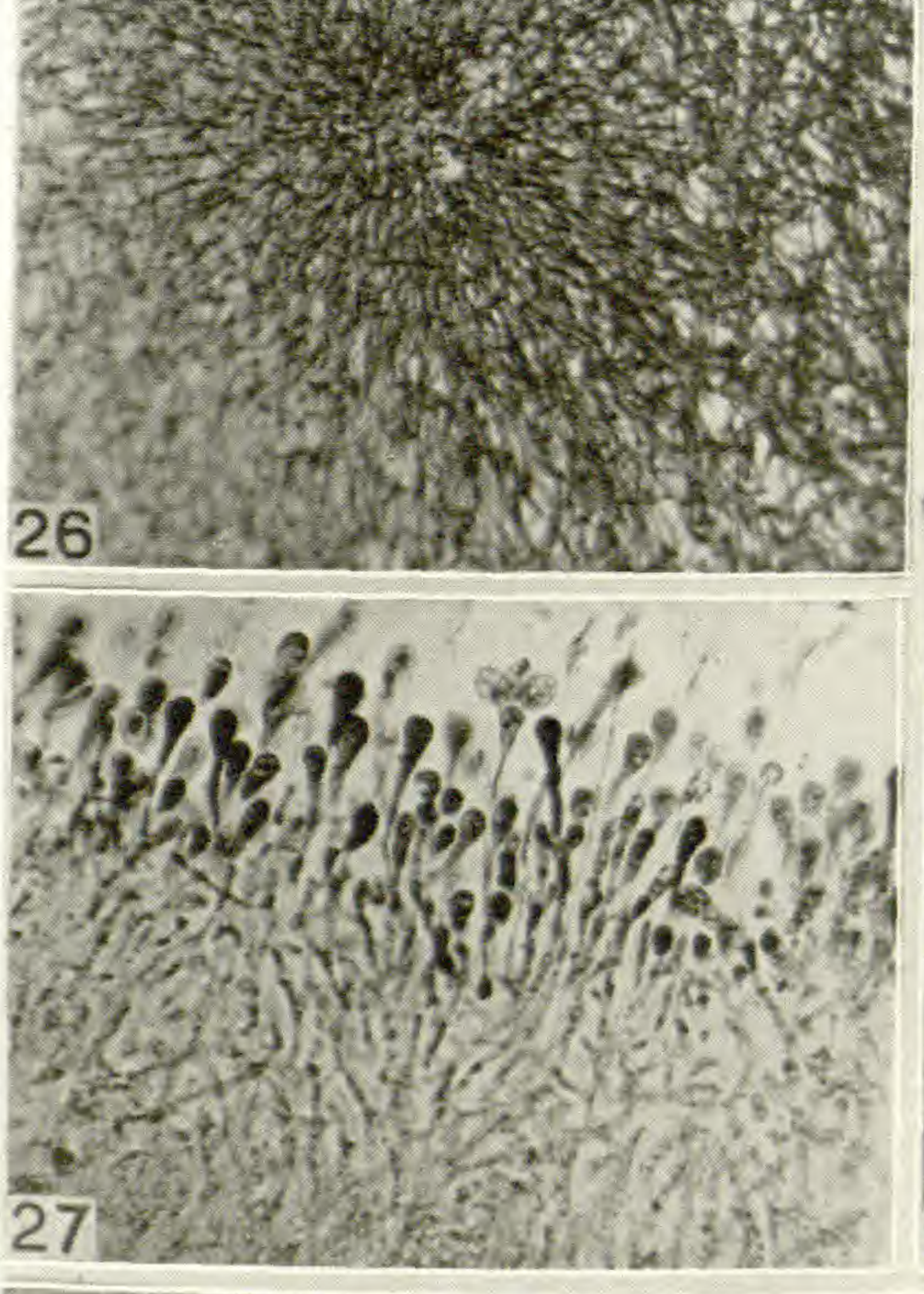
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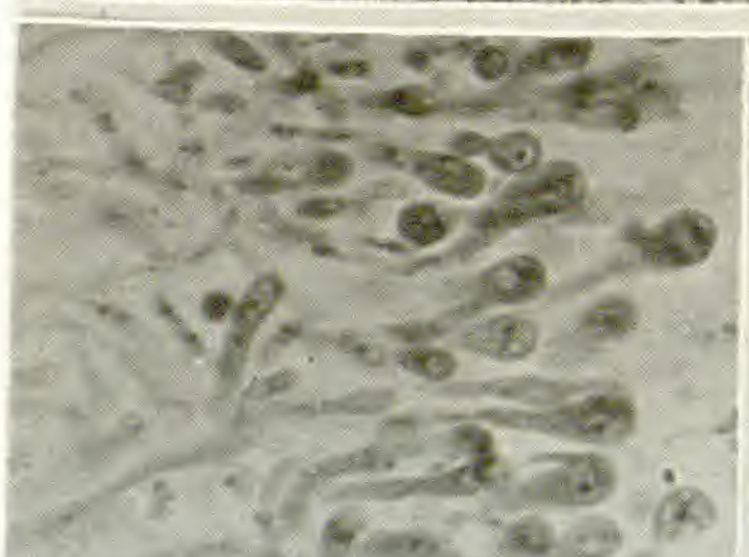
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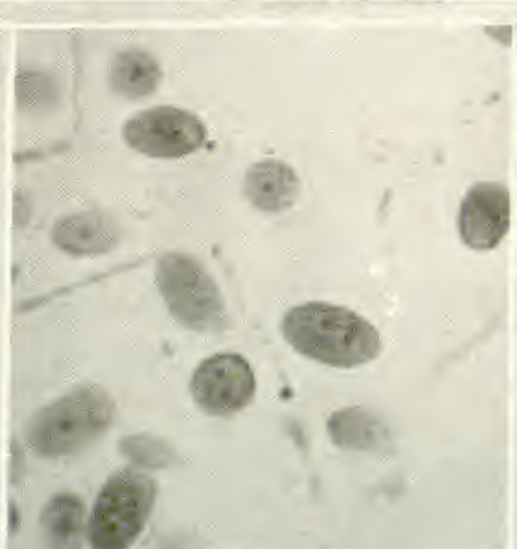
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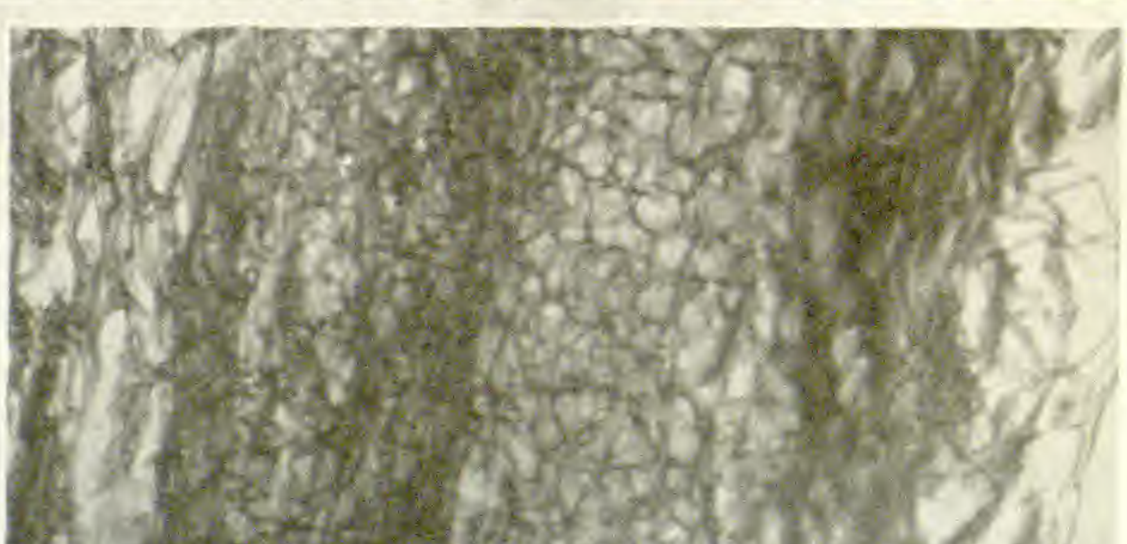

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WALKER on CYATHUS

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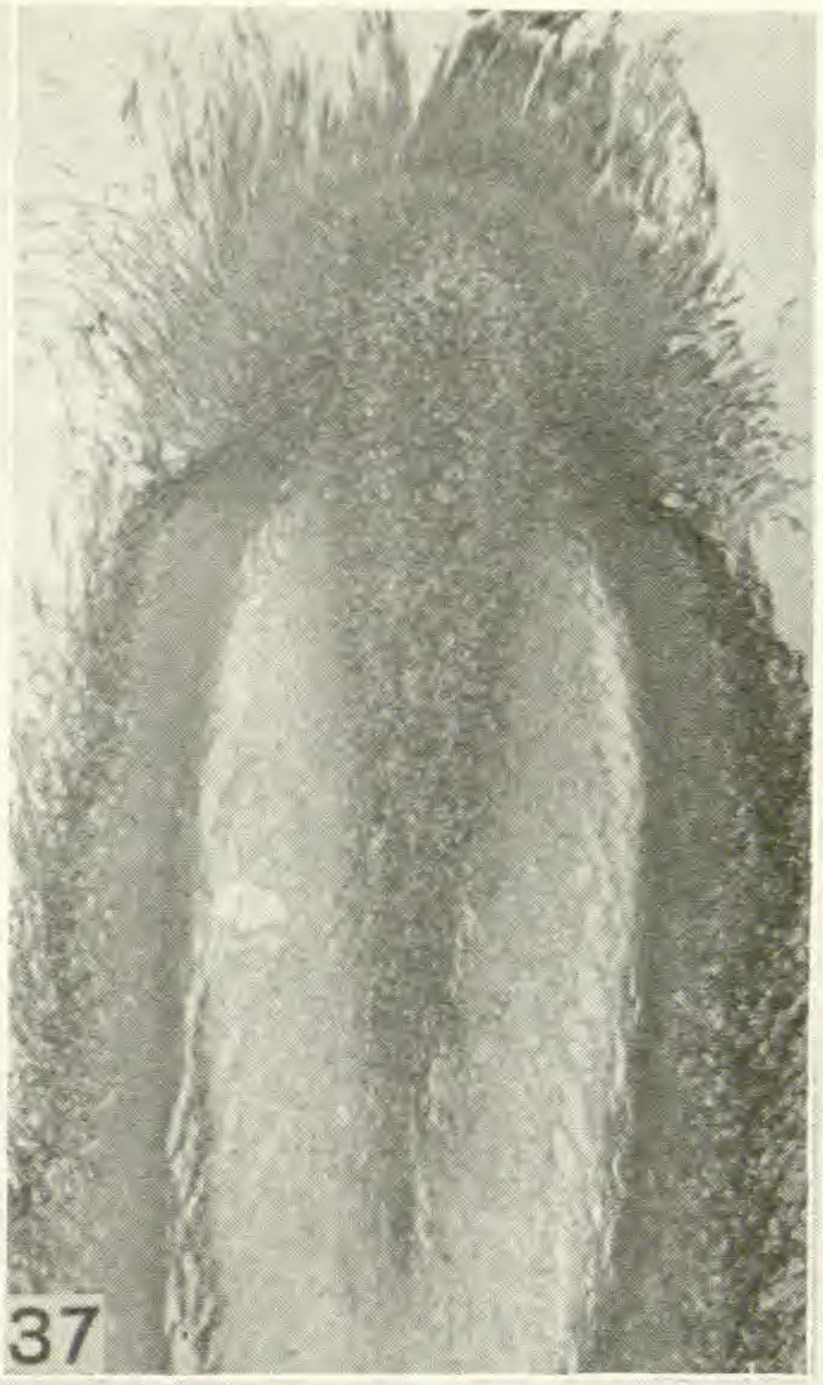
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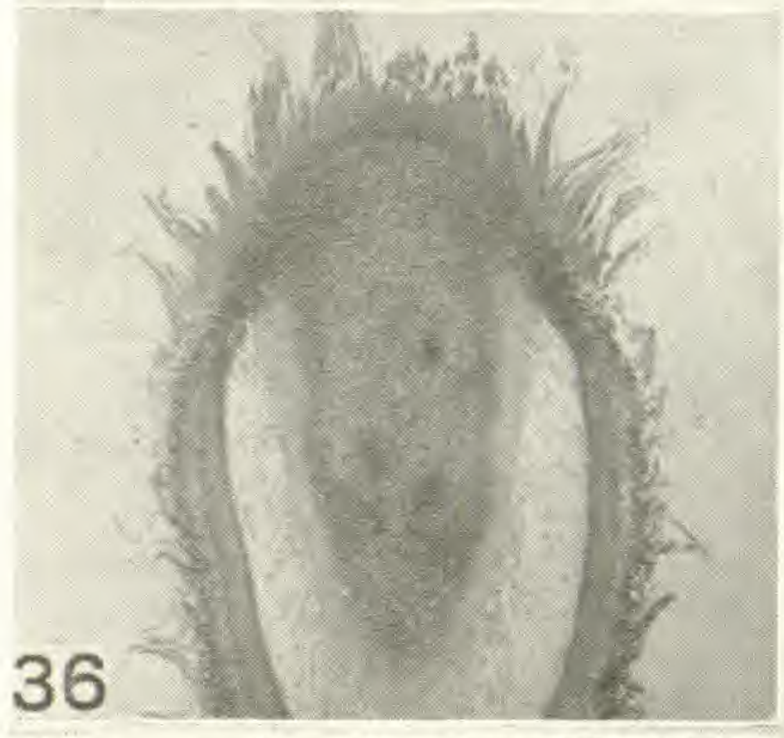
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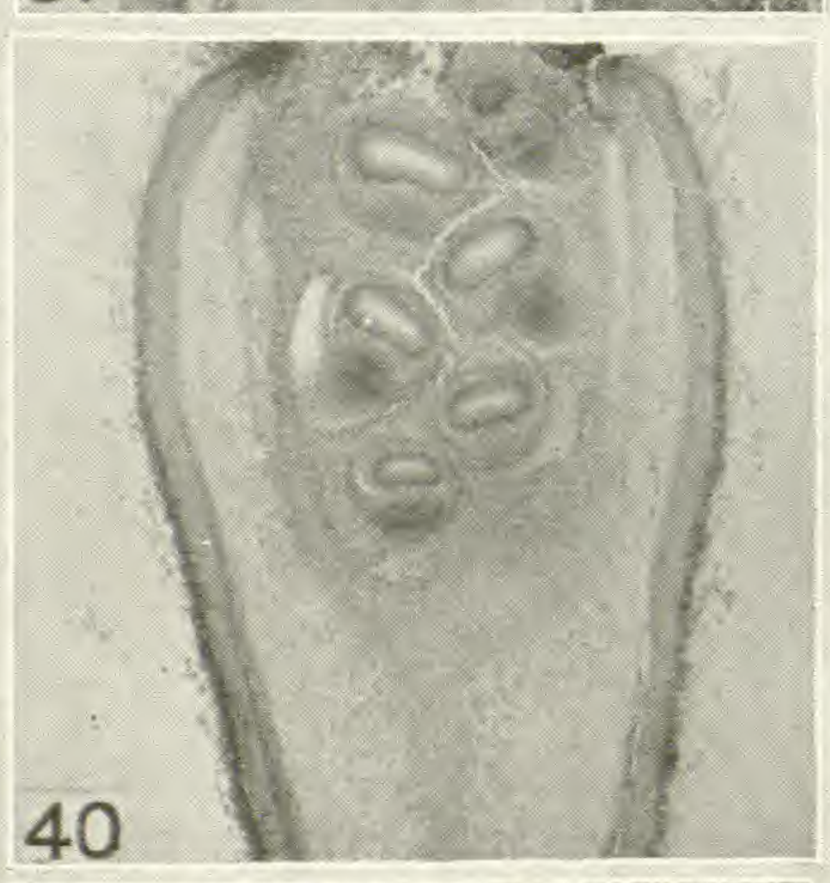
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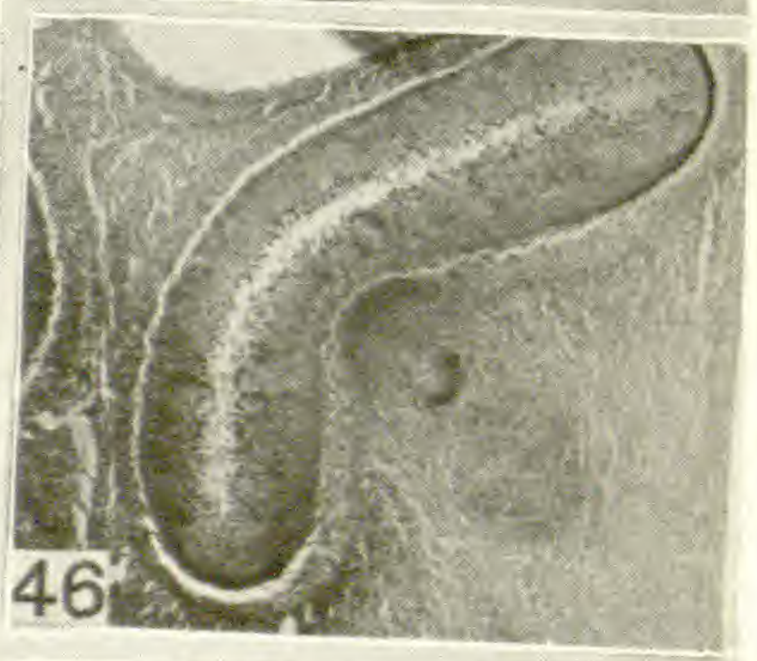
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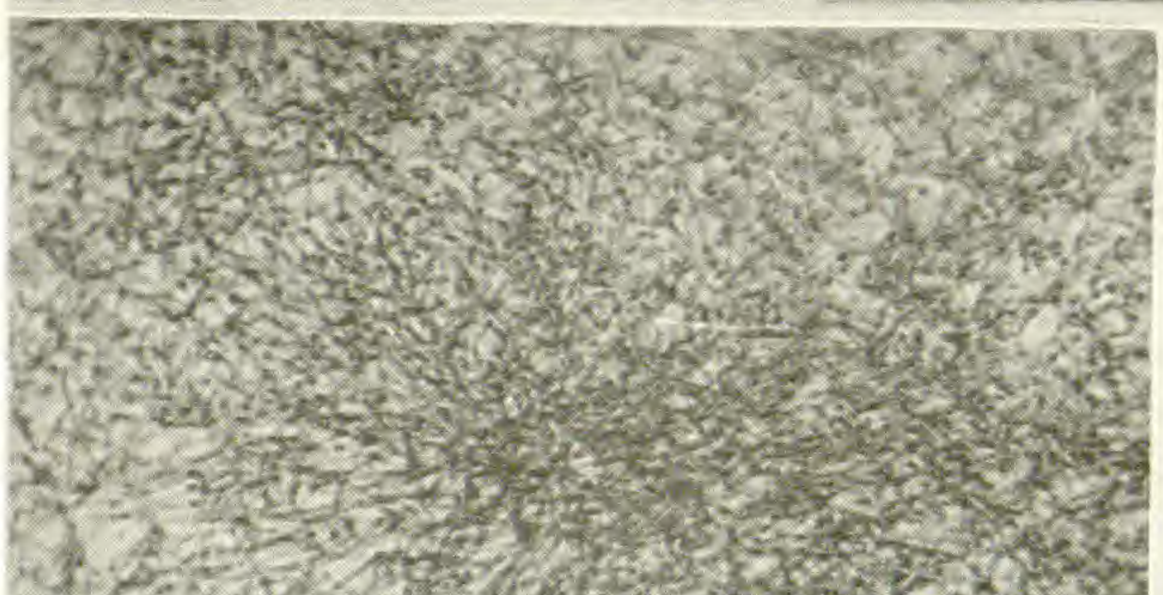
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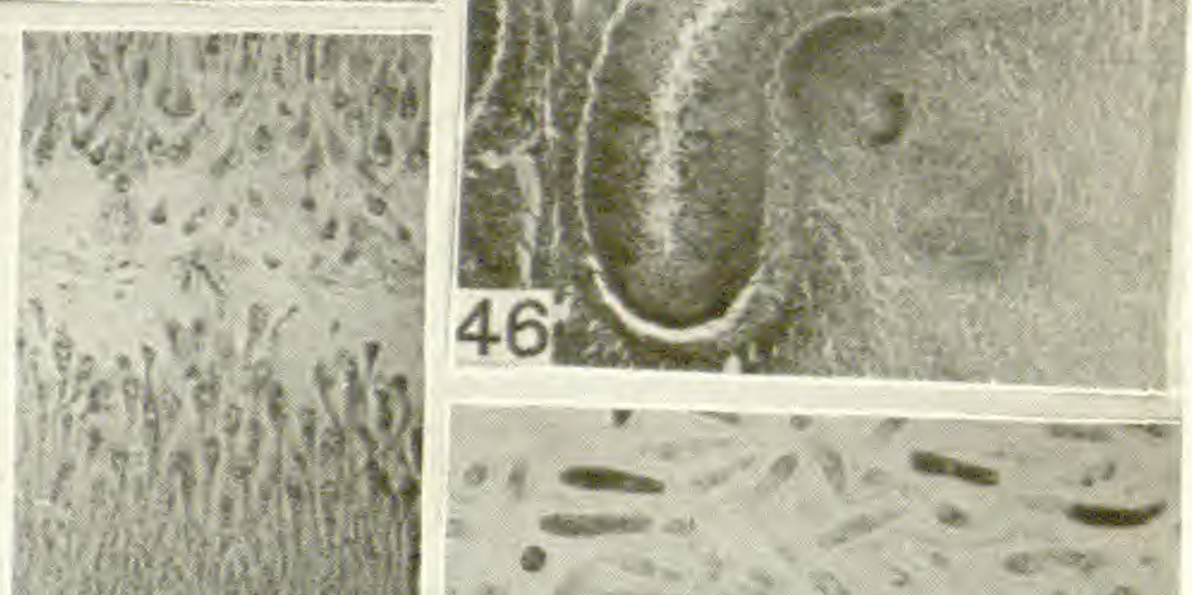
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WALKER on CYATHUS

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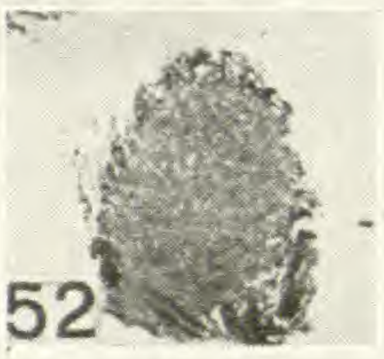
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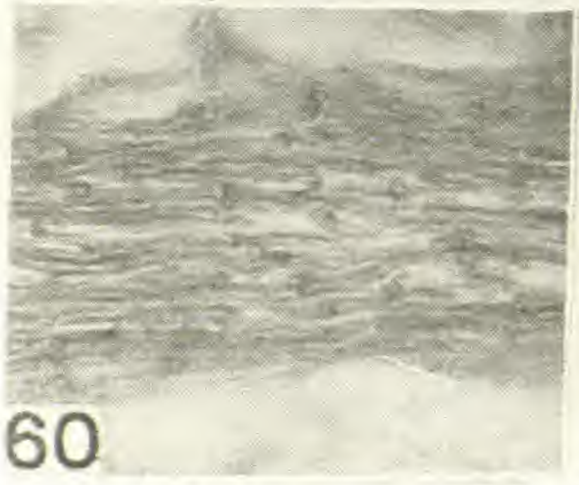
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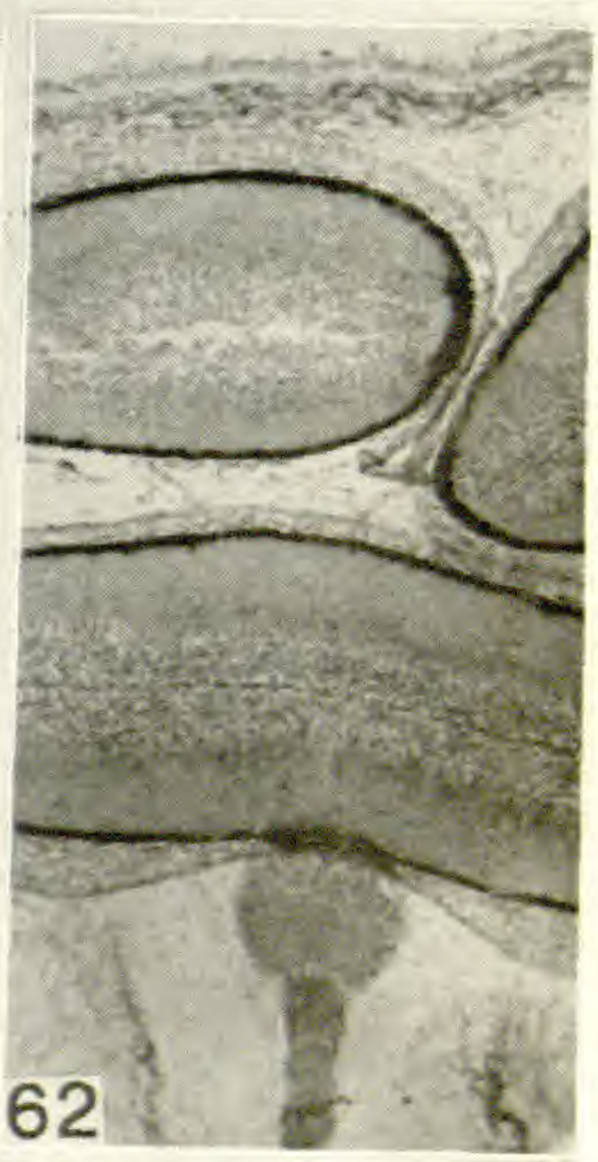
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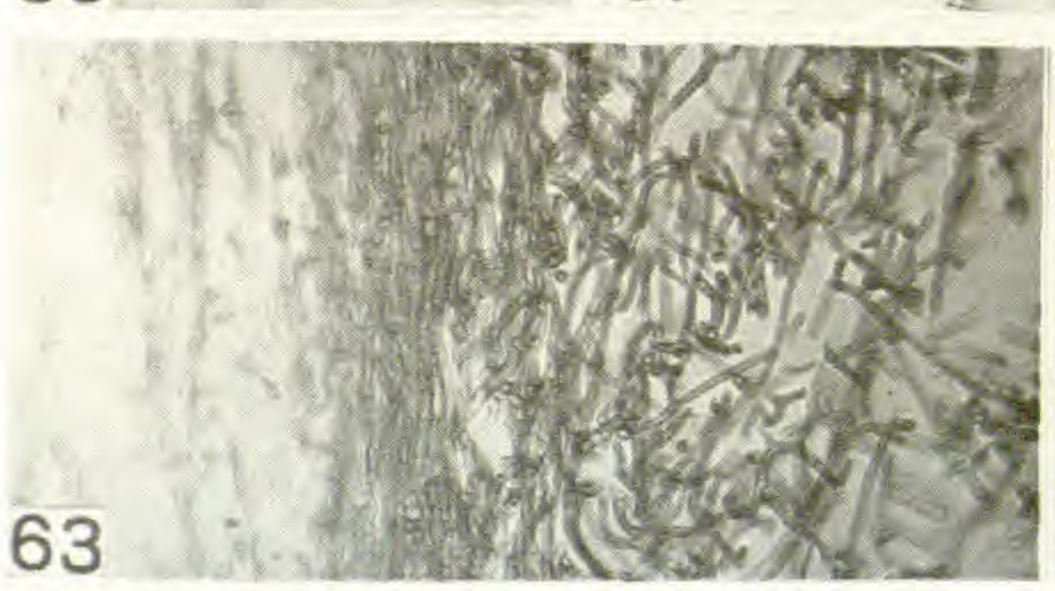
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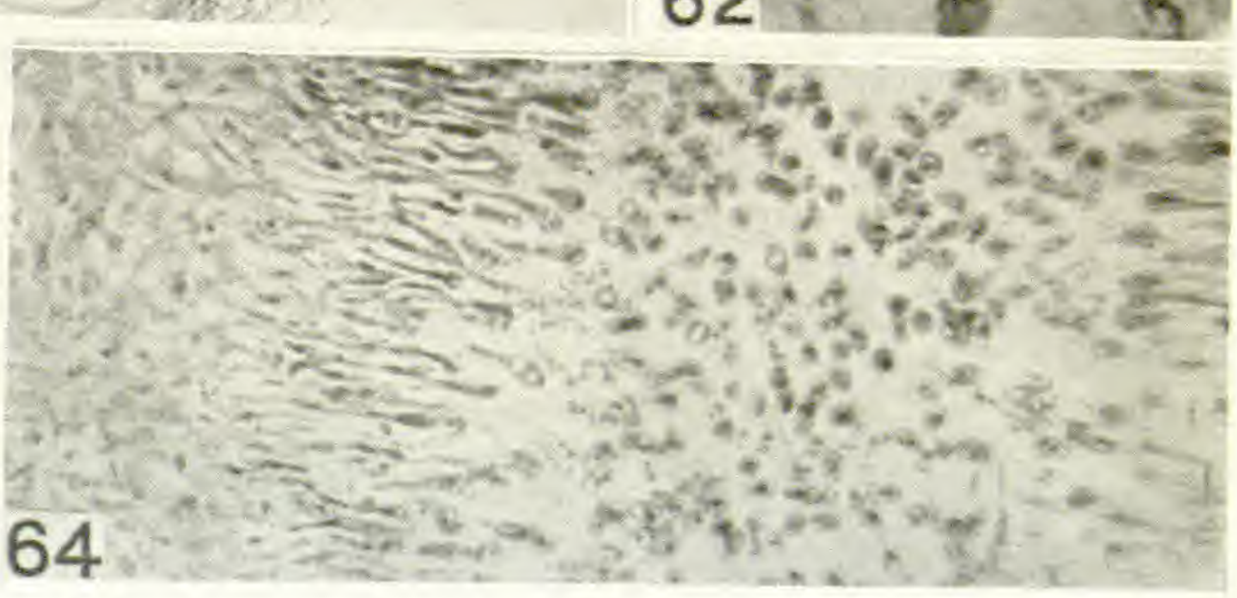
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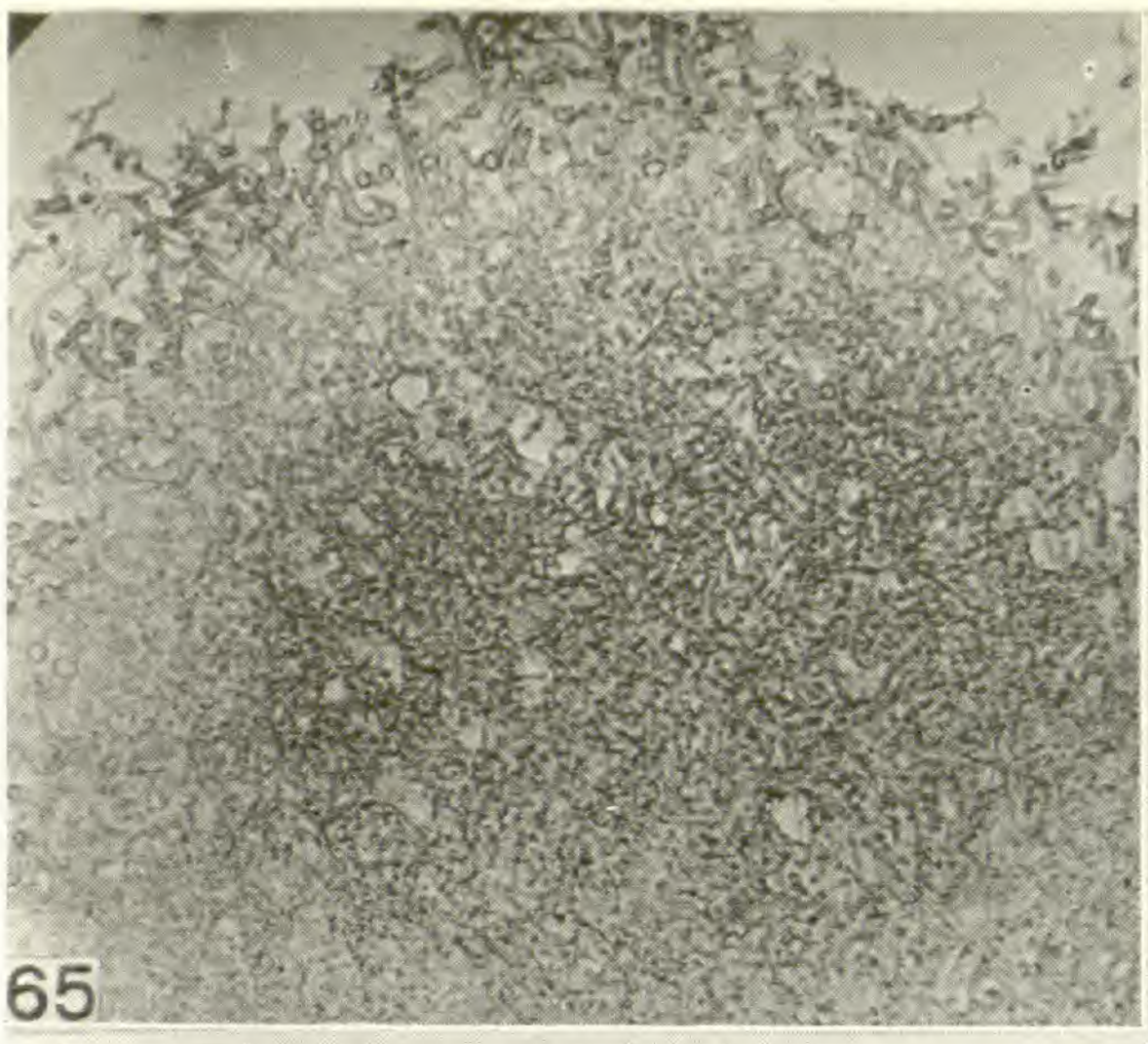
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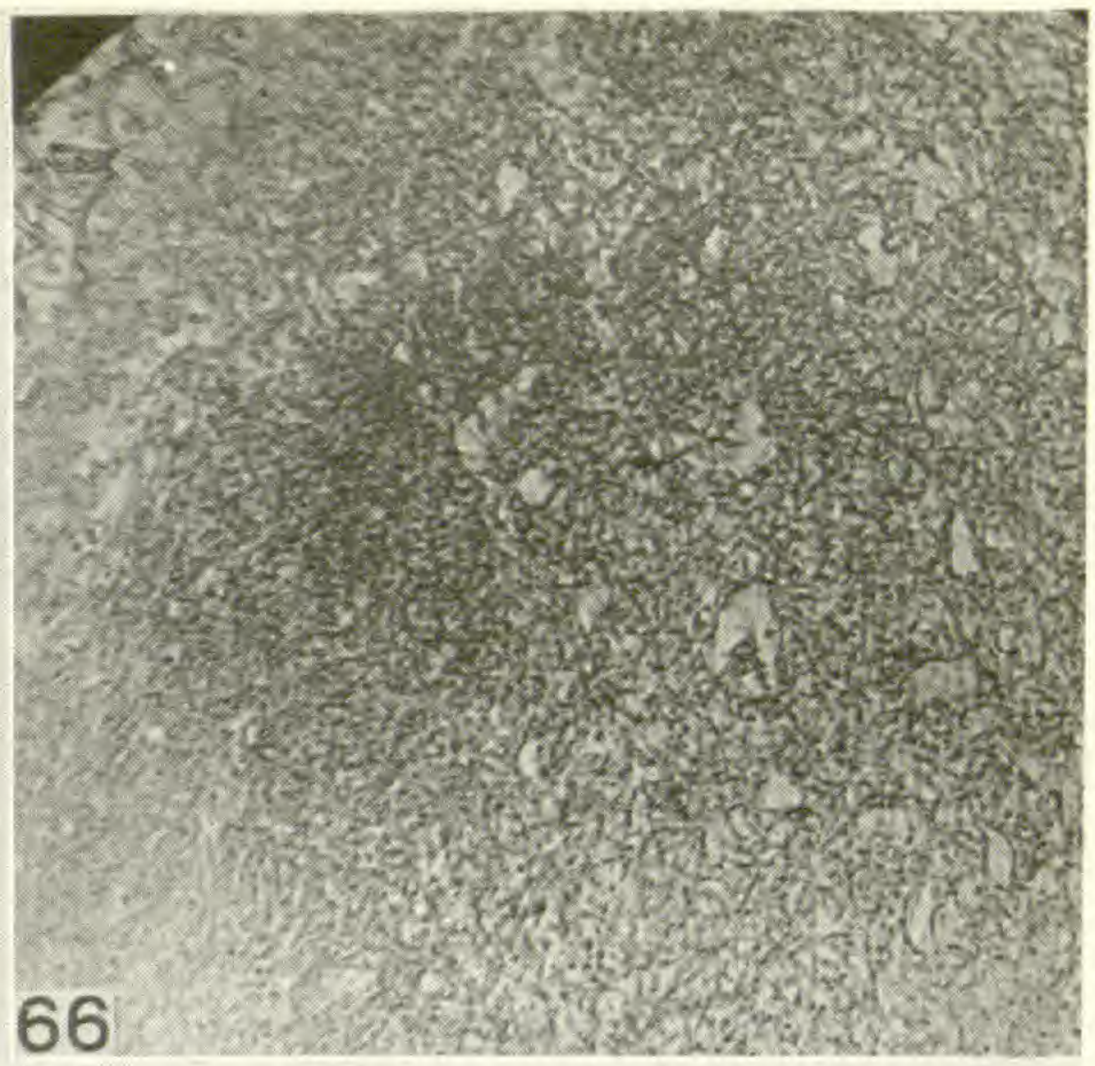
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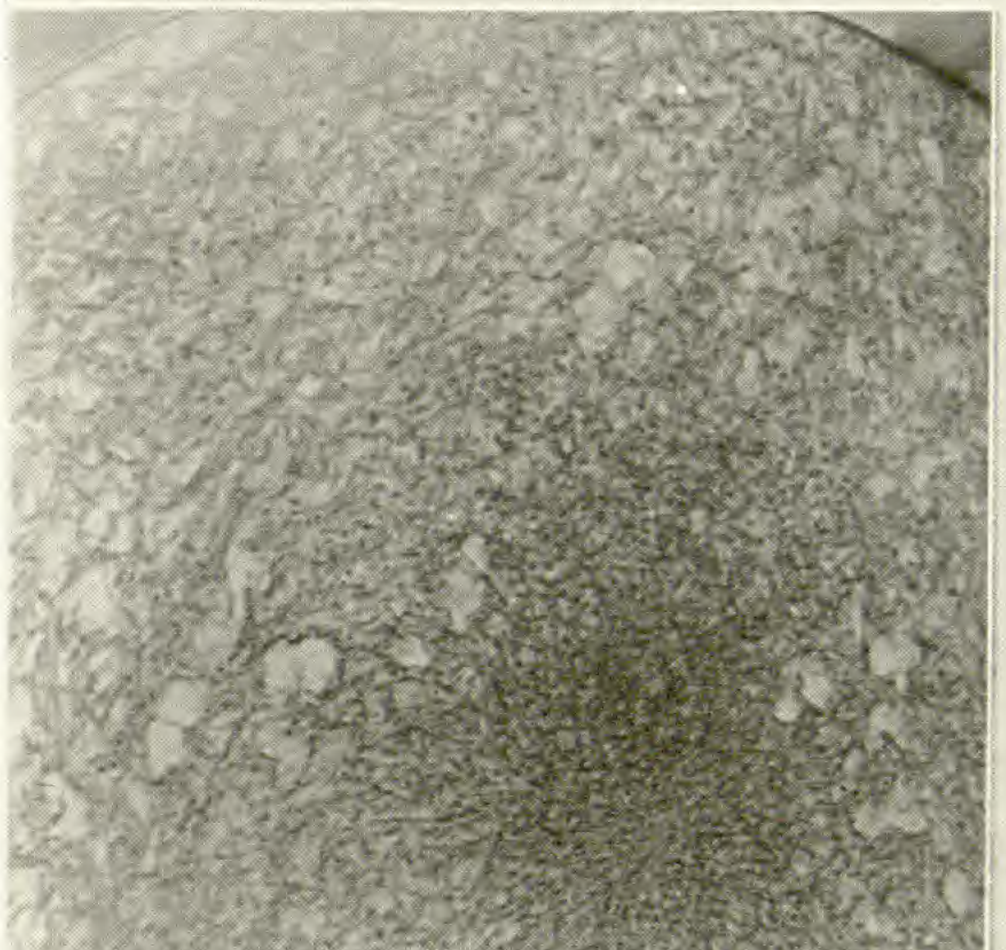
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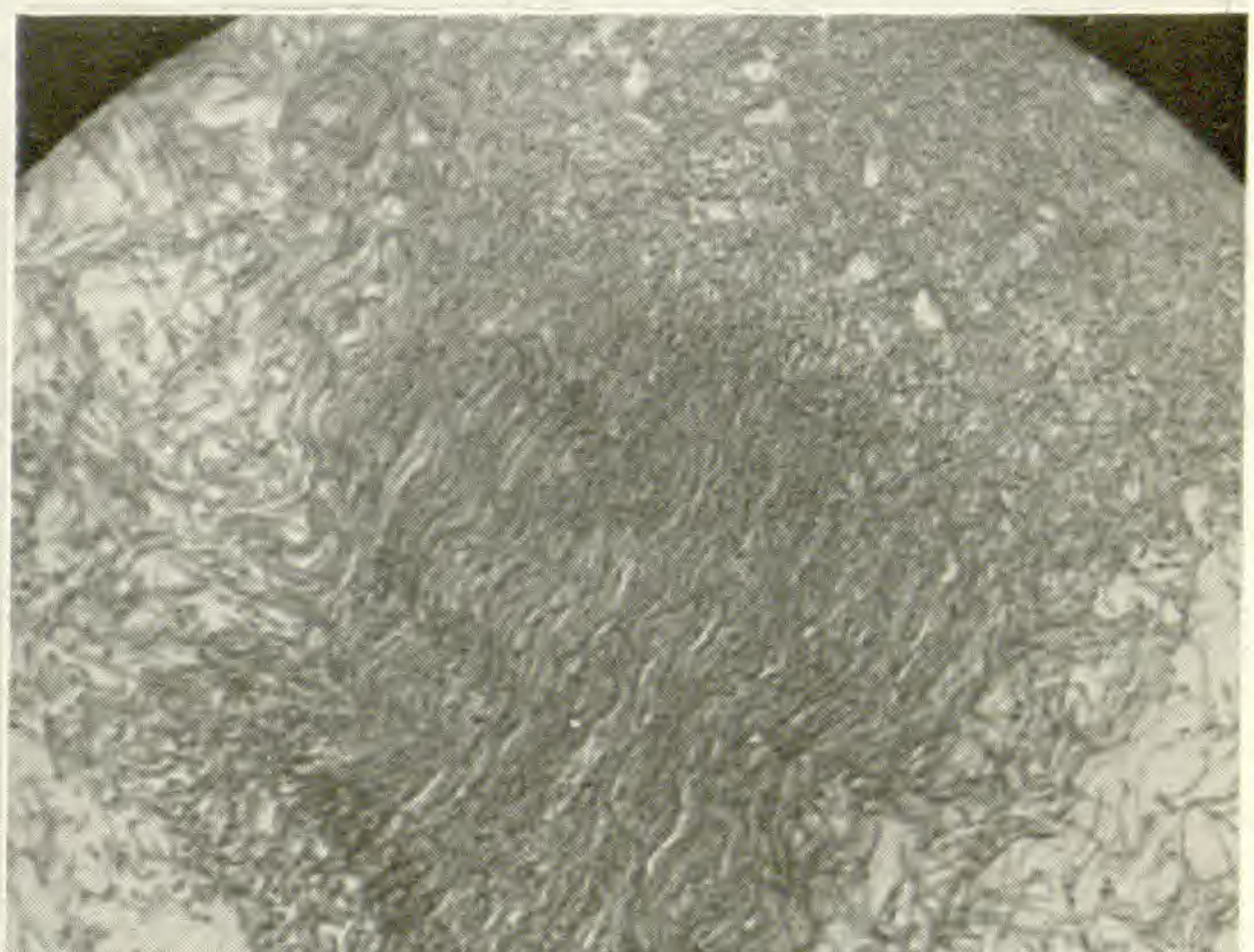
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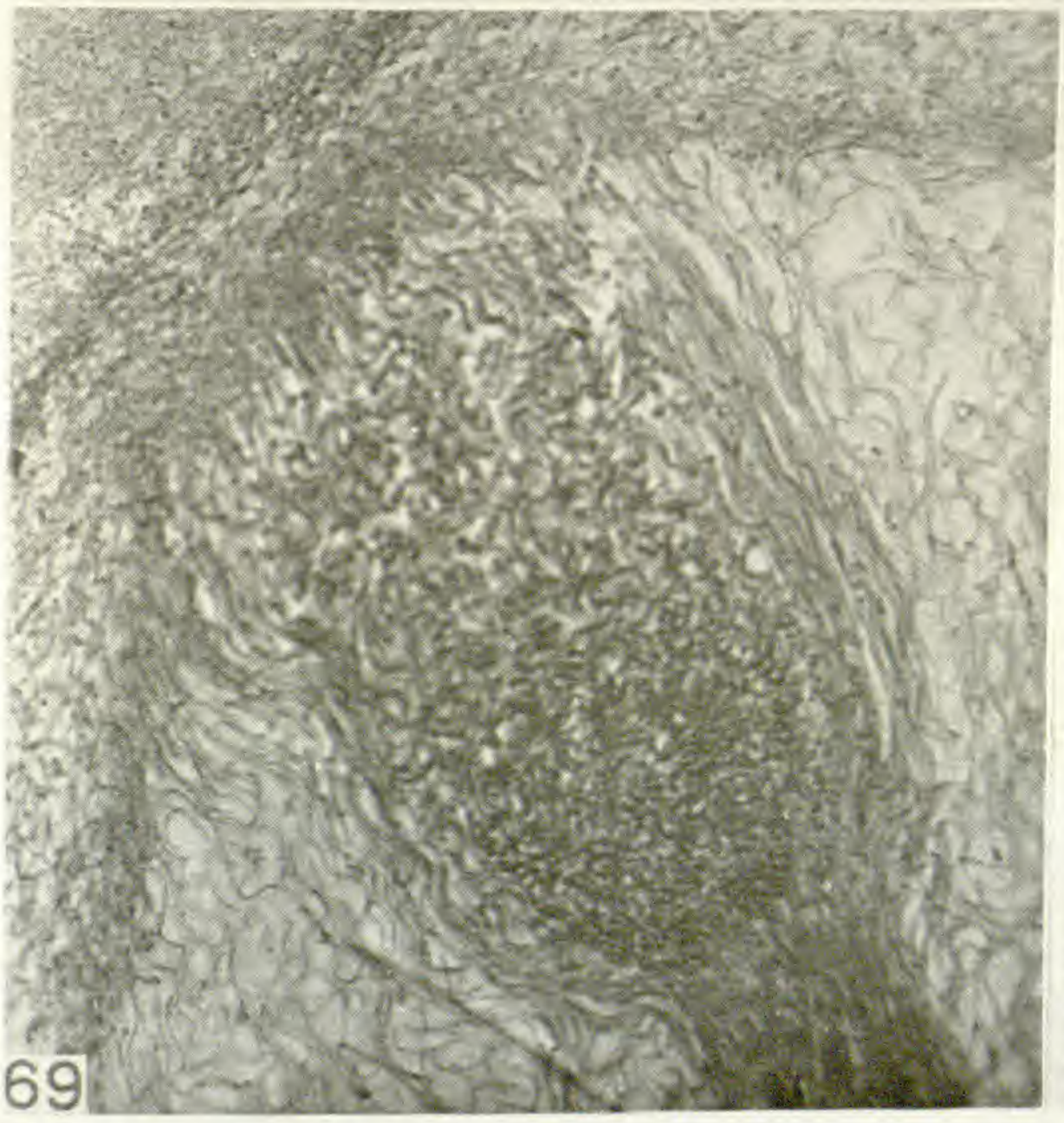
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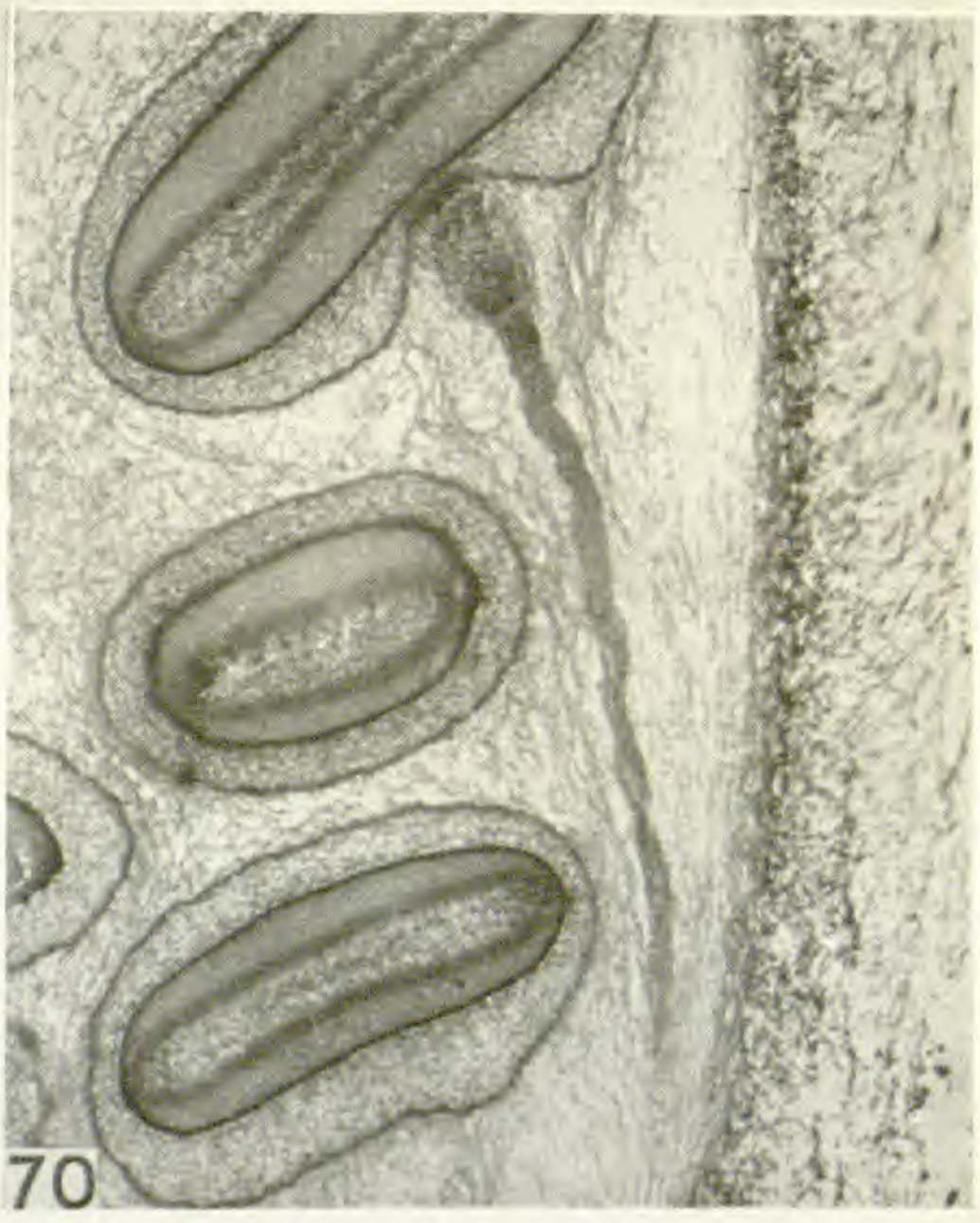
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WALKER on CYATHUS

EFFECT OF UNILATERAL MONOCHROMATIC LIGHT AND GROUP ORIENTATION ON THE POLARITY OF GERMINATING FUCUS SPORES

ANNIE MAY HURD

(WITH TWO FIGURES)

Introduction

The power of light stimuli to produce orientations and tropisms is a phenomenon which has been widely demonstrated in both the plant and animal kingdoms. Not only can unilateral illumination direct movements and growth, but in some species of plants, namely, *Equisetum*, *Fucus*, *Puccinia*, and related forms, natural white light has been found to establish the direction of the first cleavage plane of the germinating spore. Since in such cases the cell on the shaded side of the spore becomes the rhizoidal cell, the polarity of the plant is determined by light, irrespective of gravity. The primary purpose of the present investigation was to determine whether all wave lengths of light, the intensity factor being eliminated, are able to bring about this orientation and establish the polarity of the germinating spores of *Fucus inflatus*. Subsidiary studies which have been made in this connection with interesting results are (1) on that most interesting and little known phenomenon which I have called "group orientation," consisting in the orientation of the cleavage plane and the establishment of the apical and basal ends of the dividing spore by the direction of some other spore or group of spores in close proximity; and (2) on the phototropisms of the young rhizoids in monochromatic lights of equal intensities.

In reviewing the literature on biological experiments with monochromatic light, one is struck by the small number of quantitative records of the quality and especially of the intensity of the illuminations used. The ordinary light filters used to obtain monochromatic light transmit not only those wave lengths which predominate and give the color to the screen, but also other parts

of the spectrum, the presence of which can be detected only by a spectroscopic analysis. For example, certain results are frequently ascribed to blue light, with no record of just what range of the spectrum is used, nor what wave lengths other than the predominating ones are acting.

Another source of inaccuracy has been the neglect or oversight of the great variation in the intensity or quantity of radiant energy transmitted by the color screens. Biological experimenters for the most part have failed to take into consideration the fact that the quantity as well as the quality of the light stimulus varies with the different colors, and that the former variable must be eliminated before results can be attributed to differences in wave length alone. In some cases the importance of differences in the intensity factor has been recognized, but no method was known to the writer whereby the different colored lights could be compared as to their amounts of radiant energy (10).

There have been, of course, several methods devised by means of which the relative intensity of monochromatic lights can be measured. The first investigation in which the attempt was made to get monochromatic light of known wave length and equal energy was that of BLAAUW (1). He used glass color screens, and states that the lights transmitted were equal in intensity when measured with a Weber photometer. The accuracy of this method can be seriously questioned.

The next exact work of this nature was done by KNIEP and MINDER (11). They used a blue and a red color screen, and a green solution, with sunlight as the source of light. The wave lengths to which each was transparent were known; and the energy behind each was determined by means of a thermopile and d'Arsonval galvanometer. The interference of the long heat rays was prevented by inserting a water layer in a parallel-sided container between the thermopile and the source of light.

DAY (5) obtained light of known wave length by means of a spectrum from Nernst glowers, formed by a carbon bisulphide prism and cut down by a diaphragm with narrow vertical slits which could be adjusted so as to allow any desired region of the spectrum to be transmitted. In this adjustment a spectroscope

was used to determine the exact range of wave lengths passing through the slit in each of the four illuminations used (red, yellow, green, and blue). DAY measured the intensity of each with a Boys radiomicrometer, and balanced them by varying the number of glowers employed in the lamps. Thus there was one glower for the red light, two for the yellow, and three for the green and blue.

LAURENS (12), in an investigation of the reactions of amphibians, employed these same methods and the same apparatus for the quantitative analysis of the monochromatic light he used, and balanced them similarly with respect to their relative intensity. GROSS (9) also used the same methods in determining the reactions of arthropods to monochromatic light. MAST (14) measured the different effects of monochromatic light by the orientation of organisms in a field in which two differently colored beams crossed at right angles. He used spectral regions of known energy and wave length. The method of obtaining the distribution of energy is not described. PARR (15) did quantitative work on the response of *Pilobolus* to different wave lengths, using apparatus of the type employed by DAY and LAURENS.

An instrument has been devised by MACDOUGAL and SPOEHR (13) which measures the total radiant energy of any light in terms of its dissociation effect on a photosensitive substance. This is measured by a galvanometer. The advantages in the use of this "photoelectric cell" are said to be its extreme sensitiveness to the wave lengths of the blue end of the spectrum, and the fact that its action in light is "more nearly that of the organism than that of any other light measuring instrument available."

There have been, therefore, three exact methods worked out for biological experiments to obtain a quantitative analysis of light stimuli, namely, those of KNIEP and MINDER, DAY, and MACDOUGAL and SPOEHR. The interesting apparatus devised by PATTEN (16), whereby a quantitative measurement of the reactions of organisms subjected to two beams of light of different intensity is obtained, might be mentioned here. The measurement is in terms of the angular deflections from an initial path of locomotion. The same methods might be applied to work with colored lights.

A quantitative measurement of the greater effectiveness of one spectral region over another of equal intensity might be measured by the angular deviation of the path of a motile organism from a line perpendicular to a line connecting the two sources.

All these methods, with the exception of those of KNIEP and MINDER, involve special apparatus often not easily available. For many problems simpler methods will accomplish the same ends. For the present investigation a method has been devised whereby color screens of known wave length transmission are used, and their relative intensities measured by means of a thermopile and galvanometer, and made equal by adjusting the distances from the light source.

Apparatus and methods

As biological science becomes more exact, with the tendency to reduce the expression of natural phenomena to mathematical formulae, it is obviously essential to define stimuli of all sorts quantitatively. Indefinite or incomplete records of light stimuli can no longer be attributed to the lack of means of measuring them, because access to a spectroscope and thermopile makes it possible to analyze any light qualitatively and quantitatively.

There are two methods of obtaining monochromatic light for biological experiments, namely, the projection of a spectrum upon the organisms, or the use of filtered light passed through a color screen. The former is theoretically the better for exact work, but technical difficulties, such as limited dispersion and low intensity, make it impractical for many investigations. Light filters of glass are the most convenient means of securing approximately monochromatic light when unilateral illumination is desired. Ordinary color screens transmit too wide a range of wave lengths for exact work, and at present there are very few whose light is of sufficient homogeneity. The best is the Wratten filter screen, which consists of a dyed gelatine film between two glass plates. MACDOUGAL and SPOEHR have described some colored glass screens designed by them for biological work, but the range of wave lengths to which each of them is transparent is considerably greater than for some of the Wratten filters.

In the experiments to be described, Wratten light filters were used, each of which was fitted as a window in the end of a dark box. Each transmitted only a narrow range of wave lengths, but together they embraced the whole of the visible spectrum. The wave lengths to which each screen was transparent were determined by testing the light transmitted by each with a direct vision spectroscope with a wave-length scale attached. Thus the quality of the light stimulus acting in each box is accurately known. The dark boxes were 10×13 cm. and 8 cm. high, in one end of each of which a hole was cut so that one of the light filters, 5×5 cm., might be fitted into it. The boxes were made light-tight with tightly fitting covers, and were painted black inside to guard against reflections within the box. The dishes used for the cultures were either the ordinary Petri dishes or special dishes made of microscope slides cemented together with zinc cement so as to make shallow oblong dishes 7.5×2.5 cm. and 1 cm. deep. It was at first deemed necessary to use such flat-sided dishes in order to prevent possible complications from reflected and refracted light in the curving sides of round dishes, but later it was found that the same results were secured in the Petri dishes. In order to expose more than one dish behind each screen so that none would be shaded by another, a rack was made to fit inside the box with cleats projecting inward from the ends so that three dishes could be slipped into it, one above the other. The light, entering through the screen at the end of the box, fell equally on the one exposed side of each of the three dishes. The rack containing the dishes could easily be lifted out and carried to the microscope for examination without disturbing the material under investigation.

The source of light first used with the filters was the electric arc. The advantage of this light over any other artificial light is that it gives all the wave lengths of the visible spectrum, so that all the filters could be used in each exposure, insuring identical conditions of temperature, constancy of illumination, etc. The disadvantages are several. In the first place, the intensity is constantly changing as the carbons burn and the arcs get longer, and the lessening of the intensity may not be the same for all the wave lengths. In the second place, fluctuations in the current

cause large variations in the intensity. In the third place, unless an automatically adjusted arc is available, it is necessary to adjust the carbons by hand every 5 to 15 minutes, and, when an 8 hour illumination is desired, this entails considerable inconvenience. The experiments were begun with this light, however, and the results on rhizoidal phototropisms in monochromatic lights made equal in intensity were obtained with it.

The spectroscopic analysis of the light passing the screens determines definitely the quality of light entering each box. It is at once evident that the quantity or intensity of light behind filters placed at equal distances from the source varies, both because the intensity of light transmitted by the different screens is different, and because the different colors are not radiated by the arc with equal intensity. This being the case, differences in results obtained behind the screens could not be attributed to differences in the quality of the light stimulus alone.

One of the simplest means of comparing the radiant energy of colored lights is by the use of the thermopile and sensitive galvanometer. The thermopile is very sensitive to the energy of any ether vibrations, whether they be the longer infra-red or so-called heat rays, or the shorter actinic rays of the spectrum. The method developed for eliminating the intensity variable in the use of the filters employed for these investigations consisted in finding the distances from the arc at which each box with its colored window should be placed in order that the intensity might be equal in each case. These distances were those at which the deflections of the galvanometer were equal when the thermopile was exposed to the arc screened by each filter in turn.

It seems necessary, on account of the questions which have been raised during the course of this work, to state that the thermopile is equally sensitive to the energy of the red and of the violet ends of the spectrum, and is, therefore, an accurate measure of the total amount of light acting behind each color screen. The difference between heat and light is only a matter of wave length. The thermopile measures light in terms of the electric current produced by the difference in temperature of the exposed and unexposed junctions; but it does so by virtue of the fact that the energy

of whatever vibrations fall upon it, be they long and therefore heating in their physiological effect, or short and therefore perceived as light, is converted into heat energy upon being absorbed by the exposed junction of the thermopile. In other words, the light of the blue end of the spectrum produces an electromotive force much less than that of the infra-red, but no less measurable.

The instruments used in the energy calibration of these screens were a Hilger thermopile with junctions of bismuth and silver, and a moderately sensitive galvanometer (d'Arsonval). An electric arc similar to the one later used in the experiments themselves was the source of light. The thermopile with the open end screened by the red filter was exposed to the light until the galvanometer indicator reached a maximum deflection, which ordinarily took about 30 seconds. The number of divisions through which the spot of light reflected from the galvanometer mirror was displaced on the scale was noted. This was repeated six times, and the average deflection recorded. The other filters were then used in turn to screen the thermopile, and thermopile and screen moved to such a distance from the arc that the displacement of the galvanometer indicator was approximately equal in each case to that produced by the red filter. This distance was also found for the thermopile when screened by a piece of clear glass, which represented the distance of the control from the source. For the experiments the quantity of light used could be varied for the whole set of screens by multiplying or dividing these distances by the same number, and the intensity in all the boxes would remain equal. The actual amount of light in meter-candles can be measured by means of a photometer. Then from the law of inverse squares, namely, that the intensity of light per unit surface varies inversely as the square of the distance from the source, the intensity at any distance from the arc can be computed.

The calibration of the set of screens was repeated seven times, or until satisfactory checks of the distances were obtained. With some thermopiles of less rapid action than the one used here, it is impossible to get results by waiting for the galvanometer indicator to come to a steady state. In such a case the deflections produced by exposure to the light for equal intervals of time can be compared.

A series of measurements for five second exposures agreed very well with those obtained by the other method.

The absolute intensities of the light behind the colored screens were measured with a Sharp-Millar photometer.[1] Since at the prescribed distances all are equal to the white light control, there remained only to measure the intensity of the arc at the distance of the control. For the experiments the distances obtained in the calibration were divided by four, so that the intensity of the arc with the photometer 85 cm. away was measured. It was 2050 meter-candles. This must be corrected for the absorption of the light by the glass of the filters. To get this "absorption coefficient," the intensity of a light was measured both with and without a screen of clear glass equal in thickness to that of the filters. A Lummer-Brodhun photometer was used for this determination. It was found that glass 1.5 mm. thick absorbed 12 per cent of the light falling upon it. To obtain the intensity of the lights as actually transmitted by the light filters, it was necessary therefore to take 88 per cent of the reading of the photometer (2050), which was 1804 meter-candles. Of course the light stimuli acting on organisms in water in the culture dishes were still less, owing to absorption by the glass of the dish and of the water.

TABLE I

DISTANCES AT WHICH INTENSITIES OF LIGHT FROM AN ELECTRIC ARC TRANSMITTED BY WRATTEN LIGHT FILTERS ARE EQUAL

Filter no.	Wave lengths in Ångstrom units*	Color	Distance from light in cm.	Intensity in meter-candles
70.....	6600–7000	Red	320÷4=80.0	1804
71.....	6200–6800	Red	275÷4=68.7	1804
72.....	5900–6200	Orange	230÷4=57.5	1804
73.....	5600–5900	Yellow	250÷4=62.5	1804
74.....	5200–5600	Green	280÷4=70.0	1804
75.....	4700–5200	Blue	250÷4=62.5	1804
76.....	4000–4700	Violet	250÷4=62.5	1804
Control.....	4000–7000+	White	340÷4=85.0	1804

* Professor E. P. LEWIS of the Physics Department of the University of California very kindly made these wave-length determinations.

The lack of agreement between these values and the energy curve of the spectrum is probably due mainly to the peculiar

[1] I am indebted to Mr. W. C. POMEROY of the Physics Department of the University of California for these determinations.

absorption of the filters, but also partly to the fact that they do not all transmit the same number of wave lengths.

Polarity

The power of external factors to determine the polarity of a germinating spore is, without doubt, the power to orient the spindle of the first dividing nucleus, if, as in the case of *Fucus*, that polarity is established by the direction of the first cleavage plane. The work on such orientations is very limited, and has often yielded negative results. DRIETSCH established the polarity of sea-urchin eggs by subjecting them to pressure, the spindle forming parallel to the flattened sides of the egg. A number of investigators have found that unilateral white light will establish the polarity of the spores of some of the lower plants by causing the first cleavage plane to be formed perpendicular to the direction of the incident light. Without exception the cell on the darker side of the spore becomes the rhizoidal cell, the other being apical. Equal illumination on all sides retards or prevents germination. This has been demonstrated in *Equisetum*, *Fucus*, *Ascophyllum*, *Pelvetia*, *Dictyota*, *Laurencia*, *Cystoseira*, *Anthoceros*, *Fimbriaria*, *Gymnogramme*, and *Puccinia*. It has been proved that gravity and contact cannot establish the polarity of these spores.

The first report of this phenomenon of polarity established by light is that of STAHL (**20**), who worked on *Equisetum*. He found that the first wall is formed perpendicular to light rays striking the spores on one side only, and that if all sides are illuminated by rotating the spores on a clinostat, the formation of the wall is retarded or prevented. The cell on the shaded side of the spore becomes the rhizoidal cell. In darkness the formation of the first wall follows no rule, and the rhizoids extend in every direction. STAHL refers to earlier work on *Marsilia* and *Chara* which indicates that gravity is a controlling factor in the orientation of the first division plane.

ROSENVINGE (**19**) showed that in *Fucus spiralis* there is no relation between gravity and the first division plane, nor does contact with a solid body have any effect. He got the same orientation to light in *Ascophyllum* and *Fucus* that STAHL did

with *Equisetum*, but with puzzling exceptions. Where the spores were in groups, the cell toward the interior of the group became the rhizoidal cell; and in the lower part of hanging drops the rhizoids appeared on the upper side of the spore regardless of the light direction. He concluded, therefore, that not only light but a difference in the concentration of oxygen on the two sides of the spores could determine their polarity. He says that as a result of their respiration the water in the center of the groups of spores is less rich in oxygen, with the result that the rhizoids are formed on that side. In support of this theory is the fact that although light can determine the polarity of all the species studied except *Fucus serratus*, namely, *Ascophyllum nodosum*, *Fucus vesciculosus*, *F. spiralis*, and *Pelvetia canaliculata*, their sensitivity to light differs, and the oxygen factor or internal causes produce frequent exceptions in all but *Pelvetia*. The rhizoids of the latter species are always formed on the darker side of the spore, and this is the one species in which the egg is surrounded by an oogonial wall which might prevent any of the effects of varying oxygen concentration that can act more potently than light on the spores of the other species. ROSENVINGE quotes KNY as finding that neither light, gravity, nor contact can influence the point of origin of the pollen tube from pollen grains, but that in the neighborhood of other grains the tube will be sent out from the side away from them, on which side the supply of oxygen or nutritive elements would be greater.

FARMER and WILLIAMS (**6**) state that if *Fucus* spores are illuminated on all sides they tend to remain spherical instead of producing a rhizoid by the elongation of one of the two cells. Again (**7**) they experimented with one-sided illumination, with the usual result that most of the rhizoids originated on the shaded side of the spore and the others were turned that way. The fact that some grew out at an angle to the incident light was attributed to "the character of the egg itself."

WINKLER (**21**) found the same orienting effect of light on the spores of *Cystoseira barbata*, but failed to find any effect of a difference in the oxygen content of the water. He also said that gravity and contact are not factors in the establishment of the polarity of the sporelings. He found that it is determined during

the first four hours of illumination and cannot be changed afterward by any change in the direction of the incident light. He concludes, therefore, that light can orient the spore only during fertilization.

PEIRCE and RANDOLPH (18) performed one-sided illumination experiments with *Dictyota*, *Dictyopteris*, *Laurencia*, and *Cystoseira*, and pointed out the certainty of the action of other factors besides light, because rhizoids are formed in the dark and in all-sided illumination. They said that although WINKLER (21) suggested the possibility of stopping germination by changing the direction of light every three hours, it could not be done with *Dictyopteris*. They emphasized the possibility of influences preceding the illumination affecting the polarity. Later, PEIRCE (17) demonstrated this same phenomenon, that is, the orientation of the first cleavage plane and the establishment of the apical and basal cells by light, with spores of *Anthoceros*, *Fimbriaria*, and *Gymnogramme*.

The work of FROMME (8) on the urediniospores of *Puccinia rhamni* is interesting because it refutes the idea that the orientation of the sporelings by light is due to the power of one-sided illumination to cause an aggregation of chloroplasts. He said that in darkness the germ tube grew from any side of the spore, but that in unilateral light the tubes almost always issued from the darker side of the spore.

In order to obtain an abundance of spores for experimental work, the following procedure suggested by Dr. N. L. GARDNER was followed. The fruiting plants of *Fucus inflatus*[2] were collected at Sausilito at low tide one day and kept overnight in damp newspapers. The next morning they were dried slightly by exposing them to the air for from 15 to 30 minutes. The fruiting tips were then cut off and submerged in sea water in the culture dishes. After about 15 minutes many eggs and sperms settled to the bottom of the dish, or could be scraped off into the water, and the piece of plant was then removed. The fact that *Fucus inflatus* is a monoecious species makes it impossible to tell the exact time of fertilization, but it occurs soon after the eggs escape from the oogonial sac into the water. The sperms at this time can be seen

[2] Although the identification of this species is not certain, it is thought by Dr. N. L. GARDNER to be most probably *Fucus inflatus*.

escaping from the antheridia and swimming rapidly around the eggs, then scattering as, presumably, one of them succeeds in entering. The first cross-wall can be seen very plainly 24 hours after the cultures are started. The mucilage accompanying the eggs causes them to adhere so firmly to the bottom of the dish that it is not necessary to use solid media to keep the sporelings from being displaced when the cultures are moved to the microscope stage for examination.

The original plan for determining which wave lengths are the effective ones in the orienting action of white light upon the germinating spores was to use the Wratten filter screens with the electric arc. With this purpose in view, the set of seven screens borrowed from the physics department were analyzed as to wave-length transmission, and the distances from the arc were found at which the dark boxes with these screens as windows should be placed to make the light intensity equal in all. After repeated failures to get the spores to germinate on account of the high temperatures produced by the naked arc at the distances at which it was necessary to place the cultures, this source of light was abandoned, as was also a 1000-watt Tungsten globe for the same reason. Neither would the spores germinate when the boxes were placed in direct sunlight, as the heating effect was too strong, especially behind the red filters. The first positive results were obtained with a mercury vapor lamp behind the blue, violet, and ultra violet screens, the violet transmitting waves of 4000–4700 Ångstrom units, the blue 4700–5200. The same effect was produced in these lights as is produced by white light, namely, the first cleavage planes formed perpendicular to the direction of the incident light, and the cell on the darker side of the spore became the rhizoidal cell. This effect was not produced behind the green, yellow, and red screens, behind which cultures were exposed at the same time. This experiment was unsatisfactory, inasmuch as it offered no way of proving that the other wave lengths were less capable of producing the phenomenon than were those of the blue end of the spectrum, because the blue light is so much more intense in this lamp than are the red, yellow, and green. The problem was then dropped for several months, during which time no

way was found of obtaining red light sufficiently intense which did not kill the spores by its heating effect.

Finally a set of three Wratten screens was obtained consisting of a red, a green, and a blue filter, which seemed less dense than those used before. The wave lengths to which each screen was permeable were determined by means of a direct-vision spectroscope. The experiment was then repeated, using the light from large north windows facing an open field. Thus on the bright summer days used for the experiments there was a maximum intensity of indirect light available which had no disastrous heating effect. Results were immediate and decisive. The light orientation of the axes of the young sporelings was as striking in the green and blue lights as in the control in white light (fig. 1), but entirely lacking behind the red screen where the germination and development proceeded just as they did in the control in darkness. Table II

TABLE II

WAVE LENGTHS OF LIGHT WHICH CAN ESTABLISH THE POLARITY OF GERMINATING *Fucus* SPORES IN UNILATERAL ILLUMINATION

Color of light	Wave lengths transmitted	Appearance of polarity in sporelings
Blue	4000–5000 6100–6300	+
Green	4800–6000	+
Red	5800–7000	−
White (control)	4000–7000	+

indicates that from the limits of the visible blue to somewhere in the green of the spectrum wave lengths of light can establish the polarity of *Fucus* plants, while from this point in the green to the boundary of the red they cannot (figs. 1, 2). Just where, in the green, light ceases to be effective could not be determined with these screens.

The intensities of these colored lights were not equal, but the red, as in any daylight source, was many times stronger than that of the green or blue, and therefore it is all the more significant that with so much greater energy the long red rays cannot produce the reactions of the shorter blue ones. We can say definitely that

FIG. 1.—Effect of unilateral illumination with white light or its effective components on germinating *Fucus* spores, rhizoids growing from darker side of spore and extending parallel to direction of incident light; note groups of 2, 3, and 5 spores for which some chemical stimulus originating in activities of adjacent spores proved stronger than light stimulus, producing phenomenon of group orientation; ×50.

FIG. 2.—Group orientation of *Fucus inflatus* spores germinated in darkness; ×50

the power of light to orient the first cleavage planes of germinating spores and to cause the cell on the darker side to become the rhizoidal cell is dependent on the reactions within the spore initiated by the short actinic rays; that the long red rays, even though their intensity be so much greater, are ineffectual.

This response of the spores to light stimulation requires but a very low intensity of white light or its effective components. Spores left in open dishes in the laboratory rarely fail to orient themselves in a most conspicuous way with respect to the direction of the windows, nor do they require bright light. The phenomenon is just as evident in the more dimly lighted cultures left on the tables farthest removed from windows. Every culture showing this orientation, however, has more or less frequent exceptions to the general rule. Every worker on this problem has reported such exceptions, and they have been explained by assuming the existence of an inherent polarity, which as a rule is overcome by the stronger light stimulus. The fact that in absolute darkness germination and normal growth are as rapid as or more so than in light also points to an inherent polarity which is evidenced only in the absence of the stronger orienting agents.

Phototropism

WINKLER (21) first showed that the young rhizoids of *Fucus inflatus* are negatively heliotropic. With the apparatus designed for the light polarity experiments just described, several questions concerning this phenomenon were easily answered. These were: (1) what wave lengths are responsible for the turning away from a source of white light? (2) is the intensity of the illumination a factor, or is the phenomenon controlled only by the wave lengths acting; in other words, what is the rôle of the quality factor apart from the quantity of the light stimulus? (3) do all lights which have any effect at all produce the same negative tropism produced by white light?

Although the light emitted by the electric arc was too low in intensity after passing through the dense filters to establish the polarity of the young *Fucus* plants, it was noticed very early in the investigation that behind the blue and violet filters this light

was strong enough to cause all the rhizoids to turn sharply away from it. Experiments were started, therefore, to determine whether those behind the filters transmitting the longer wave lengths would not also show this effect, the intensity of all the lights being kept equal. The spores were germinated in small dishes in darkness, and allowed to grow until the rhizoidal cell had divided at least once. Cultures were then placed in the seven dark boxes behind the original set of seven Wratten filters, and these boxes placed at such distances from the naked arc that the intensity of light behind each was 1804 meter-candles (table I). The illumination was continued 6–7 hours. The next day the cultures were examined to see which ones showed the characteristic negative phototropism. It was found in every case that only those illuminated by the blue and violet light had been so affected, those behind the other filters having their rhizoids unbent, continuing in the direction in which they had started, just as did the control in darkness. With all the intensities the same (1804 meter-candles), therefore, wave lengths capable of producing the negative phototropism so commonly seen after white light illumination are those of 4000–5200 Ångstrom units, all the others having no effect.

TABLE III

WAVE LENGTHS WHICH WITH AN INTENSITY OF 1804 METER-CANDLES PRODUCE A NEGATIVE PHOTOTROPISM IN *Fucus* RHIZOIDS

Filter no.	Color	Wave lengths in Ångstrom units	Distance from arc in cm. at which intensities are equal	Appearance of negative phototropism
70.....	Red	6600–7000	80	−
71.....	Red	6200–6800	68	−
72.....	Orange	5900–6200	57	−
73.....	Yellow	5600–5900	62	−
74.....	Green	5200–5600	70	−
75.....	Blue	4700–5200	62	+
76.....	Violet	4000–4700	63	+
Control.....	White	4000–7000	85	+

The same experiment was tried with sunlight as the source of illumination. The young plants were exposed behind the filters all day in a south window. The same results were obtained as

when the arc was used. Then the experiment was repeated with diffused light. The boxes were placed in an east window for 8 hours. Again the rhizoids in the blue and violet light showed the response, but in addition a considerable but much smaller number were affected in the same way behind the green filter. Later the experiment was repeated with the second set of three filters with which results were obtained in the experiments on light polarity. These, as explained before, were less dense than the first set, and were used with the light from a north window. Here the negative phototropism appeared behind the green and blue filters, but not behind the red. Thus the same filters whose light was found to have the power of establishing the polarity of the germinating spores were also the ones which produced the rhizoidal phototropism (table II). As explained previously with reference to these filters, the intensity of the ineffective red was many times greater than the shorter but more powerful wave lengths. Just where between 4800 and 6000 Ångstrom units the rays cease to be effective it is impossible to tell with these filters, but with the first set it was found that the phototropism occasionally occurred behind the green filter with a range of 5200–5600 Å.U. It is probable, therefore, in view of the fact that in these latter experiments the blue and violet rays of 4000 to 5200 Ångstrom units never failed to produce the phenomenon at equal or less intensities, that the tropic power of light gradually decreases from the violet and blue toward the red end of the spectrum, losing its power at ordinary intensities somewhere around 5600 Å.U. It remains to be seen whether the still longer rays can be made to produce the same effects by increasing their intensity.

Only the growing tips of the rhizoids are sensitive to light. This usually results in a sharp angular turn if the direction of illumination is changed through 90° or 180°. As pointed out by LOEB and others, such tropisms are probably due to the difference in the speed of the chemical reactions going on in the two sides of the growing tip. The first protuberance of the germinating spore is not affected by light striking it from the side; and if it is so illuminated during the early stage of elongation of this cell, the first bend occurs at the cross-wall separating it from the next

rhizoidal cell. In other words, the direction of the first protuberance is at right angles to the first cleavage plane, whatever the direction from which it is subsequently illuminated. After the first cross-wall has formed in the rhizoid, however, a change in the direction of illumination results in an angular bend at the tip.

Group orientation

The preceding experiments have demonstrated the power of light to establish the polarity of germinating spores of *Fucus inflatus*. Yet another factor was found to exert an orienting influence on the spindle no less potent than that of light, that is, the proximity and direction of other germinating spores. ROSENVINGE (**19**) described this most striking and interesting phenomenon in other species of *Fucus* and in *Ascophyllum*. The first cross-wall forms perpendicular to the direction of the adjacent spore or group of spores, or, if the spore be one of a group, perpendicular to the direction of the center. The cell toward the interior of the group, or toward the source of the stimulus in the case of more isolated cells, becomes the rhizoidal cell (fig. 2). For want of a better term I have called this phenomenon *group orientation*. It is best studied in cultures germinated in darkness, and hence free from orienting effects of light.

This phenomenon is as conspicuous in groups of 2, 3, or 4 eggs as in masses of 50 or 100, so long as they are within the distance of each other through which the stimulus is effective. This distance is usually 0.2–0.3 mm., but occasionally spores as much as 0.5 mm. apart have shown the mutual orienting influence. If there are only two spores concerned, the first cleavage planes are often parallel, and the rhizoids, growing from the inner cells, meet tip to tip. In the small groups of five or six the rhizoids, all growing toward the interior, make rather symmetrical starlike designs. In the larger groups or masses of spores the phenomenon is made evident by the fact that no rhizoid is ever found taking a direction away from the group. Although the finding of many groups of eight lying together just as they escaped from the oogonial sac, and beautifully oriented with respect to each other, suggests an inherent polarity established by the relative positions of the eggs in the

oogonium, the phenomenon appears just the same if these groups are stirred up with the point of a needle before being germinated, so that the original positions are entirely changed.

The direct cause of such orientations is probably the same as that responsible for those produced by light. At least the results of the stimulations are identical, and it seems probable that the ultimate factor is the relative rate of the oxidations proceeding along an axis of the spore. It follows that the energy of light might determine this oxidation gradient in unilaterally illuminated cultures, but in those germinated in darkness the oxygen content of the water on the different sides of the spore, being more exhausted on the side next to other growing spores, might disturb the equilibrium and produce the same sort of a gradient. ROSENVINGE advanced the theory that the phenomenon was produced by a difference in the concentration of oxygen or of nutritive substances on the two sides of the spore. He thought the rhizoid forms on the side toward the center of a group or toward another egg, because as a result of the latter's metabolism the water on that side is less rich in the active substance than on the outer side of the spores. WINKLER, however, working with *Cystoseira barbata*, found that a difference in oxygen concentration which he produced artificially had no such effect on the spores.

This phenomenon, group orientation, is found in cultures germinated in light as well as those in darkness, although not so conspicuous in the former, because the light may be the stronger stimulus for many spores which in its absence would be affected by the orienting stimulus from adjacent spores. Yet the fact that it is always found in cultures germinated in unilateral light, although limited to those spores and groups of spores within a very short distance of each other, shows that within this distance the influence of neighboring spores is stronger than that of light, at least of lights with the intensities of those used in these experiments. In other words, no light was powerful enough to overcome for the more closely grouped spores the chemical stimuli originating in themselves. The relative number of spores oriented by light depends therefore on the intensity and wave-length composition of the light source and the distribution of the spores in the culture. In many

cultures only the more isolated spores will be oriented by light, the others all showing strongly the group orientation. The spores show the greatest individual differences in their relative sensitiveness to light and to the group stimulus. Of two spores lying within about 0.3 mm. of each other, one might be entirely oriented by the adjacent spore, while the other, apparently like it, would show only the action of the light stimulus. In many cases two such spores would seem to show a resultant effect of the two stimuli, so that both would be half turned toward each other, with both rhizoidal cells showing a tendency to take a direction away from the light at the same resultant angle (fig. 1).

The substance or condition originating in the activity of adjacent spores which has so powerful an effect in orienting the first cleavage plane and in determining which cell shall become the rhizoidal cell has no power to cause any chemotropism of the rhizoids after they are started. No rhizoid has been found to have its direction modified by the presence of other spores adjacent to it. In the absence of any light stimulus the rhizoids continue in the direction that they take originally from the spore.

Discussion

CHILD'S (3, 4) metabolic gradient theory seems to offer the most satisfactory explanation of the power of environmental factors to establish the polarity of germinating spores. He has demonstrated in many marine plants and animals the existence of "axial susceptibility gradients" which he considers due to a decreasing rate in the metabolic processes from the apical to the basal or posterior end. Such a gradient would be established by differences in the rate at which oxidations and other reactions proceed, and by this disturbance of the equilibrium of the physiological mechanism would determine the basal and apical ends of the organism. CHILD'S explanation is as follows:

> Since extended experiment with the lower animals indicates that the degree of susceptibility to cyanides and to many other agents and conditions is in a general way, and within certain limits, a rough measure of metabolic activity or of certain fundamental metabolic processes, probably primarily the oxidations, these axial differences in susceptibility in the algae are regarded as indicating the existence of axial metabolic gradients. . . . . In the final analysis such a gradient is not self-determined by some sort of organization,

but arises as the result of the differential action of factors external to the protoplasm, cell, or cell mass acted upon. If, for example, an undifferentiated cell or cell mass is stimulated at some point by the action of a factor external to it, the resulting increase in metabolic activity is not limited to the region immediately affected, but a wave of change spreads or is transmitted over or through the protoplasm with decreasing energy, intensity, or physiological effectiveness, until, if the mass be large enough, it becomes inappreciable at a greater or less distance from the point of origin.

In the case of determination of the polarity of a spore by the direction of its illumination, it might be said, therefore, that a gradient is established within it by virtue of the fact that the oxidation reactions proceed more rapidly on the side receiving the greater amount of light energy. This side would become the apical end, if CHILD'S supposition is correct that the higher rate is toward the apex, or head, and that in the posterior parts, or in the rhizoids of algae, the rate is least. Thus it might be said that the disturbance of equilibrium within the spore due to the reception of unequal amounts of light energy over its surface produces an oxidation or metabolism gradient which establishes the polarity of the young plant. The spindle is oriented in some unknown way, and the less active of the two cells resulting from the first cleavage is the rhizoidal cell.

Why only the rays of the blue end of the spectrum should have this action is not clear. Possibly the cells exercise a selective absorption such as that described by BOVIE (2) as possessed by *Paramoecium* when acted upon by ultra violet light. Then the differences in the effects of monochromatic lights on *Fucus* spores would be due to differences in penetrating power rather than to "any action specific of wave length." Possibly the energy becomes available to the cell for its effect on oxidations through some photosensitive substance which responds only to the actinic rays.

CHILD has recently found a metabolic gradient or oxidation gradient in these germinating spores, and he finds that the region of highest susceptibility, which he takes to be the region of highest oxidation, is at first at the rhizoidal end, suggesting that the original effect of the light is an inhibition of reactions on the exposed side of the spores. A letter written to the author regarding this point contains the following:

As regards *Fucus*, I found the situation very interesting and very similar to that which exists in some of the lower animals. The egg shows a gradient as soon as polarity is determined, but as you suggested, it might be the region of highest susceptibility, which I believe to be the region of highest oxidation rate, is at first at the rhizoidal end. This makes it look as if the effect of light might be a differential inhibition rather than a differential stimulation. After five or six days, however, the susceptibility of the rhizoid decreases, and at the same time a new region of high susceptibility begins to appear at what is to be the apical end of the thallus, and this becomes and remains the most susceptible region of the plant, and from it a gradient of decreasing susceptibility extends basally to the base of the thallus. It looks as if the outgrowth of the rhizoid represented a rather brief period of high rate of oxidative activity, which soon slows down, and then the apical region of high rate arises, just as a bud, previously inhibited, arises or begins to develop when the activity of the growing tip which inhibited it is decreased. Of course these are at present merely suggestions by way of interpretation of the observed facts.

As for group orientation, it and the effect of light may have a common physiological basis. In both phenomena the controlling force may be gradients of increasing oxidation rates, but with different factors responsible. In the case of the light effect it may be the available energy speeding or retarding metabolic reactions where the wave lengths acting are such that they can be absorbed by the active substances of the cell; or in the case of group orientation, it may be available oxygen or other nutritive substance varying in amount on two sides of the spore as the result of the metabolic processes of adjacent spores.

We must conclude, however, that the attempts at partial explanation of these experiments and observations are far from satisfactory. The application of the oxidation gradient theory can only account for the later aspects of the polarity phenomena, the determination of the apical and basal ends of the germinating spore. The orientation of the first cleavage plane determined by orientation of the spindle of the dividing nucleus is visible evidence of forces existing within the cell, and the control of those forces by light energy and by chemical stimulation in a manner of which there is no hint, and the mechanics of which must remain obscure for the present.

Summary

1. A convenient method for obtaining monochromatic lights of equal intensity is the use of the thermopile and galvanometer

to obtain the relative intensity of the light transmitted by accurate color screens, and the adjustment of the distances of these screens from the light source such that the deflections of the indicator on the galvanometer scale are equal for each exposure of the thermopile screened by the light filters in turn.

2. The effective wave lengths in the establishment of the polarity of *Fucus* spores, the result of whose use for unilateral illumination is the same as that produced by white light (the orientation of the first cleavage plane perpendicular to the direction of the incident light with the cell on the darker side of the spore becoming the rhizoidal cell) are, with the intensity of strong diffused daylight, the shorter rays of the blue end of the spectrum of approximately 4000–5600 Ångstrom units. There is some evidence that ultra violet light can produce the same effect.

3. The negative phototropism of the rhizoids in monochromatic light is also primarily a function of the quality of the light, since, with equal intensity of illumination, the wave lengths of the red end of the spectrum are without effect, while those of 4000–5200 Ångstrom units produce the same phototropism produced by white light.

4. The term "group orientation" is suggested for the phenomenon of the orientation of the first cleavage plane of a dividing spore with reference to the position of adjacent spores, such that it is perpendicular to the direction of the center of a group or of a single spore within the effective radius, with the subsequent development of the cell on the side toward the source of stimulus as the rhizoidal cell.

5. This group orientation reported in other species is a conspicuous phenomenon in every culture of *Fucus inflatus*, the stimulus acting in such orientations being so strong that when spores are separated by as much as 0.2 mm. and often more, light stimuli as a rule fail to overcome it.

6. The chemical stimulus which orients the direction of the first cleavage plane and determines which cell shall become the rhizoidal cell in group orientations has no power to cause a chemotropism of the rhizoids.

I wish to acknowledge with gratitude the encouragement and assistance of Dr. T. H. GOODSPEED, under whose direction this investigation was carried on; and the helpful suggestions of Dr. C. M. CHILD and Dr. N. L. GARDNER; and also of Dr. E. F. LEWIS, who has placed the facilities of the physical laboratories at my disposal.

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CROWN-GALL OF ALFALFA

O. T. WILSON

(WITH PLATES VII–X)

Introduction

The crown-gall of alfalfa has been known in the United States for only a few years, the first published record of its occurrence in this country being in 1909. It was at that time observed in California by SMITH (**32**). Since then it has been reported from Arizona (**23**), Oregon (**26**), and Utah (**27**). It is probable that it also occurs in other western states. The disease has been known somewhat longer in other countries. PATOUILLARD and VON LAGERHEIM (**28**) in 1895 published the earliest record of its occurrence in connection with an outbreak in Ecuador. In 1902 MAGNUS (**21**) described the general features of the disease and recorded its presence in Germany. It has also been reported from England (**29**), and Italy, Sweden, and Switzerland (**10**).

The lack of detailed information makes it difficult to estimate the economic importance of the disease. Old and powerful stands were killed in the fields of Ecuador (**19**). MAGNUS considered the disease serious at Colmar in Alsace. SALMON (**29**) advised strict precautions against its spread in England. In the United States MCCALLUM (**23**) reported the disease as serious but not widely distributed in Arizona at the time of his observations. SMITH noted it in only a few counties of California. According to O'GARA (**26**), many plants two to seven years of age were destroyed by the parasite in Oregon. In 1915 resolutions were adopted by the American Phytopathological Society (**31**) recognizing the serious possibilities of the disease and recommending its investigation by the government.

It is the purpose of this study to contribute to our knowledge of the life history of the causal organism.

Material and methods

Infected plants were secured from the vicinity of Medford, Oregon. In the winter of 1913–1914 P. J. O'GARA sent specimens,

and in the fall of the year 1914 material was received from Dr. M. P. HENDERSON. Other material was secured from alfalfa plants grown and infected in the greenhouse.

Standard histological and cytological methods were employed for the examination of the material; reference to these will be made in connection with various phases of the observations.

Observations

In 1902 MAGNUS (**21**) described the galls as branching tuberculate structures on the larger secondary roots of *Medicago sativa*. Upon examining cross-sections of the galls, he found large brown regions of irregular form, which proved to be cavities filled with resting spores of the parasite. "Thick-walled strongly encysted mycelium" was found in many of the cavities, but he did not find that the resting spores were attached to the hyphae. The amount of mycelium present in the different cavities varied; it was often entirely lacking. The hyphae were described as continuous or branched, and the protoplasm of the host cells was often completely displaced by these "wandering hyphae." MAGNUS thought that this mycelium might "awake to new life" after the winter resting period. He described the resting spores as spherical with one side flattened; he noted a colorless hyaline cell attached to the flattened side by means of a hyaline process. Many pores were found in the centers of the flattened walls of the spores. No other stages in the life of the parasite were mentioned by MAGNUS, and not all those described were figured. So far as the writer has been able to learn, no subsequent work has been published touching upon the life history of this organism, which has been classified as *Urophlyctis alfalfae* (von Lagerheim) Magnus. Figures of the galls have been published by various writers (**13, 22**), and all agree with MAGNUS' original description.

The alfalfa plants which furnished the material for these observations were several years old. Numerous galls corresponding to the structures described by MAGNUS and others as the crown-gall of alfalfa were found upon the plants (fig. 1). Free-hand sections of such galls upon microscopic examination revealed numerous brownish resting spores, like those figured by MAGNUS,

occupying irregular cavities in the tissues of the gall (fig. 8). If the spores are not scattered in sectioning they completely fill the cavities. They average 40 μ in diameter. When shaken from the cavities they appear as a glistening brown powder.

With rare exceptions the spores are spherical in form, except for the depression on one side (fig. 8). Great irregularities of form may occur, especially near the borders of masses of spores, where the host tissues apparently interfere with the natural conformation of the spores. These irregular spores may conform to the outlines of the host cell occupied (fig. 11). A group of slitlike pores in the depressed surface is normally a prominent feature of the spores (fig. 9). BALLY (3) observed similar pores in the walls of the spores of *Urophlyctis Rubsaameni*. The walls of the spores consist of two layers, the outer much heavier than the inner (fig. 6). The outer layer is yellow-brown, glassy in appearance, and brittle, as shown in sectioning. It is very resistant to stains, this quality being characteristic of resting spores of the Chytridiaceae. A positive reaction with phloroglucin indicates that it is lignified, but it does not stain with safranin. The inner layer of the wall is thin and hyaline in appearance; it responds to the zinc chloriodide test for cellulose. In this respect it is like the wall of the sporange of *Olpidium Viciae* described by KUSANO (17). It lacks the rigidity and brittleness of the outer layer. Ridges in the inner layer are of frequent occurrence (fig. 13).

In the resting condition the protoplasm of the unstained spore appears to be granular in nature and somewhat vacuolate (fig. 10). The nuclei cannot be distinguished without staining. Flemming's triple stain or Heidenhain's iron-alum-haematoxylin may be employed to bring out the nuclei. Many small nuclei are found to be scattered quite regularly through the cytoplasm of the spores (fig. 15). It is difficult to detect the structure of the nuclei at this stage; it becomes easier as the spore develops into a sporange. There is considerable variation in the response of the nuclei to stains. This is probably related to the difficulty in obtaining very thin sections through the spores. Nuclei which have been sectioned show that the chromatin is usually at the periphery, often concentrated at one side. In some preparations

a nucleole appears clearly (fig. 24). The condition of the resting spore at this stage corresponds to that found by LOEWENTHAL (**20**) in the sporange of *Olpidium Dicksonii* at the very beginning of zoospore formation, when the presence of very numerous tiny nuclei was noted. Before staining, the content of the resting spore appears hyaline and refractive in section. Only the nuclei stain deeply; about each nucleus is a clear region, the clear portions being separated by lightly stained cytoplasm.

It is only in the resting stage that the heavy-walled bodies should be called spores. From the nature of their further development they are clearly potential sporanges. The resting spores of the Chytridiaceae have frequently been called sporanges.

The development of the sporanges was first studied in Van Tieghem cells, distilled water being the medium employed. The spores undergo an immediate change when placed in water. The granular appearance gives way to one in which the cell seems to be filled with small globules of oil. This indicates the first changes leading to the formation of zoospores. If the spores are crushed at this stage, numerous globules of fat are freed which stain with Sudan III or with osmic acid. As development continues, the fat globules in the sporanges apparently become larger and less numerous (fig. 14). This condition continues until the motile zoospores are set free.

In water the wall of the sporange also undergoes an almost immediate change; a swelling of the wall on that side takes place, presumably because of the entrance of water through the pores in the depressed surface. BÜSGEN (**5**) observed a somewhat similar swelling of the wall in the sporanges of *Cladochytrium Butomi*. The sporanges become almost or quite spherical in outline. The pores become cracks, and after a time the outer layer may rupture as the inner content becomes more turgid (fig. 18). Various appearances are brought about by the protrusion of the inner layer of the wall and its contents. Sometimes the outer layer is thrown off like a cap, as BÜSGEN observed in his study of *Cladochytrium*. In the majority of cases, however, the outer layer remains intact. The whole process indicates a softening or gelatinization of the wall as compared with its previous brittle condition.

Just before the exit of the zoospores a motion of the sporange contents is visible. The fatty globules are jostled about although they do not flow together. In a spore which is not viable the fat is frequently congregated into one or two large drops, but such a spore was never seen to develop into a sporange. The motion within the sporange is followed by the rupture or dissolution of the inner wall layer, allowing the zoospores to escape through the openings in the outer layer. The difficulty of observing their direct exit is enhanced by the fact that the porous side of the sporange wall is almost always downward in the hanging drop. The zoospores escape sometimes in groups, but usually singly. Often they seem to have difficulty in locating the pores, and they may swim about in the sporange for a considerable time or even disintegrate there (fig. 19). ATKINSON (1) has observed an interesting amoeboid movement of zoospores within the sporanges of *Rhizophidium globosum* just previous to their escape. Although no such amoeboid movements were seen at this stage in my material, the alternation of resting and active periods is a common occurrence, just as was observed by ATKINSON.

In stained sections the development of the sporanges may be traced in a fairly complete series. Even in the very early stages each nucleus seems to be related to a definite portion of the cytoplasm (fig. 26). The nuclei have never been observed to occur in groups, but are rather uniformly distributed. As development continues, the nuclei decrease in number, as shown by the number present in a cross-section of a sporange. Apparently there is a disintegration of many of the small nuclei, while those which are to take part in the formation of zoospores increase in size. The structure of the latter nuclei also becomes much more clearly differentiated, and they stain with more uniformity (figs. 20, 27). The chromatin is now aggregated in knots connected by slender threads (fig. 16). In most of the preparations the connecting threads are not easily seen; in such cases one or more knots are apparent just within the nuclear membrane (figs. 20, 27); these large nuclei are the centers of zoospore formation. BARRETT (4) found in *Olpidiopsis* nuclei similar to those here described, which served as centers of zoospore formation. The transition stages

from the condition shown in fig. 15 to that in which the spores are delimited have not been clearly followed. Zoospore formation is evidently a very rapid process. With the possible exception of the cilia, the zoospores are quite fully developed before their escape from the sporange. The vacuole and nucleus are quite well defined (figs. 23, 25). LOEWENTHAL (**20**) found a vacuole and well defined nucleus in the zoospore of *Olpidium Dicksonii* before its escape from the sporange. BARRETT observed the vacuole in the zoospore of *Olpidiopsis*, but apparently did not succeed in staining the nucleus. A cleavage of the sporange cytoplasm previous to zoospore formation is apparent in well stained sections. This cleavage begins at the margin of the sporange, and works toward the central region (fig. 22). The process apparently corresponds to that described by HARPER (**16**) for *Woroninella*.

An interesting variation from this development is of quite common occurrence, but has been observed only in fixed and stained material, probably because it is masked in fresh material by peripheral fat globules in the sporange. Soon after the nuclei begin to enlarge in the stages preceding zoospore formation, one of them (sometimes several) undergoes an especially rapid development (figs. 17, 21), becoming separated from the other nuclei by a surrounding portion of denser cytoplasm. This is often quite clearly shown, even in unstained sections. A spore may be clearly delimited about this nucleus, while the other spores are yet in an early stage of development. This spore is much larger than the others formed in the same sporange. All the zoospores are alike in structure, the only marked variation being in size. The formation of several of the large spores in the same sporange is exceptional (fig. 29).

When freed in water, the zoospores exhibit a great variety of movement. Often the long cilium, which is quite clearly visible, seems to impede the movement of the zoospore, which exhibits a violent jerking motion. Irregular gyrations are common. Frequently a zoospore moves back to the sporange from which it escaped and seems to seek an entrance. At times the zoospores move very rapidly from the field of observation in a direct line. The movements become less vigorous after a time; the ellipsoid

or ovoid changes to a spherical form, and the long cilium becomes more plainly visible than before, dragging behind passively. This last described feature has been reported for the motile spores of various Chytridiaceae. Periods of activity alternate with periods of passivity, during which the vacuolated condition is very evident. The fat drop is visible only during the active periods. BARRETT (4) and BUTLER (6) have observed pulsations of the vacuoles in the spores of *Olpidiopsis* at this stage.

The fat drop is the most prominent internal feature of the active zoospore. It may be seen to shift rapidly when the zoospore is in motion. After a period varying from a few minutes to several hours, terminated by sluggish amoeboid movements, the zoospore comes to a final rest and soon disintegrates. It seems likely that the refractive body so commonly observed in the zoospores of the Chytridiaceae is a drop of fat occupying a vacuole. ATKINSON (2) observed the presence of a prominent fat drop in the zoospore of *Rhizophidium brevipes*.

Amoeboid movements of zoospores have frequently been mentioned by investigators of the Chytridiaceae. SCHENK (30) observed this phenomenon as early as 1858. DANGEARD (8) noted that the zoospores of *Chytridium xylophilum* creep like amoebae. BÜSGEN (5) observed similar movements in his study of *Cladochytrium Butomi*.

I did not directly observe the exit of the large zoospores from the sporanges, but they were seen in considerable numbers moving slowly about in the water, remaining close to the sporanges from which they had emerged. The content of these large zoospores appears to be more granular than that of the smaller ones, which latter appear hyaline in water. Very soon after their exit the large zoospores are surrounded by the smaller ones. One or several of the latter move swiftly toward a large zoospore and become attached to it. As many as five small zoospores have been seen adhering to one large spore, but in all cases only one remains attached. There seems to be no uniformity as regards the point of attachment. The small zoospore which remains attached loses its cilium just at the time of contact. KUSANO (17) reported a resorption of the cilium at this stage in *Olpidium*. After a small

zoospore has become permanently attached to a large one, the latter continues to move about for a time before coming to rest. An amoeboid form is finally assumed, and disintegration soon follows.

Fusion of zoospores has been reported but rarely in the Chytridiaceae. Probably this is because of the very limited observations upon the motile stages. FISCH (14) observed and figured a fusion of zoospores for *Reesia amoeboides*. DANGEARD (9) noted an apparent fusion in *Sphaerita endogena*. KUSANO observed a clear case of fusion in *Olpidium*, the fusing cells being similar in size. The observations of SOROKIN (33) upon *Tetrachytrium*, of ATKINSON (2) upon *Lagenidium*, and of VON LAGERHEIM (18) upon *Rhodochytrium* should also be mentioned in this connection.

The motile spores were fixed and stained upon the cover slip by the use of osmic acid and gentian violet. Each spore was found to have a short cilium as well as the long one visible in the water (fig. 28). The motion of the zoospore, after the long cilium becomes passive, is probably due to the activity of the shorter cilium. It seems possible that the uniciliate condition is not so common among the Chytridiaceae as has been thought. Care in staining and observation is necessary for a successful determination of the number of cilia. CORNU (7) found only one cilium borne by the motile spore of *Olpidiopsis*. FISCHER (15) later found two, which observation was confirmed by BARRETT (4). Cilia of unequal length on the same zoospore have been reported and illustrated for various members of the family, as for example *Sphaerita endogena* (9).

The cilia of the motile cells of the organism under consideration are attached at the same end of the zoospore to what seems to be a platelike thickening of the membrane. The manner of attachment is much more clearly seen in the larger zoospores (figs. 31, 32). The oil vacuole is near the place of attachment of the cilia (fig. 40). The nucleus lies back of the vacuole, imbedded in the cytoplasm. From the cilia to the nucleus there is a connecting, cone-shaped, apparently fibrous structure, which extends through the vacuole (figs. 31, 32). Fig. 25 shows a general similarity to the zoospore structure described by LOEWENTHAL (20) for *Olpidium*, and figured by NOWAKOWSKI (25) for *Polyphagus Euglenae*.

Fusion stages may be quite clearly followed in material fixed and stained on the slide. Fig. 28 shows two zoospores, a large one and a small one, apparently in the same condition as when freed from the sporange (see fig. 30). The nuclei of stained zoospores often appear to be within a vacuole because of their position when fixed to the slide. The larger zoospore, either before or at the approach of the smaller one, may put out one or more projections in the direction of the latter (fig. 33). Fusion may take place at the apex of such a projection (figs. 34, 36). The two nuclei may be seen within the larger body following the fusion (fig. 35). The fusion of the nuclei has not been observed. Figs. 37 and 38 possibly indicate stages following a fusion succeeded by a division of the fusion nucleus. Many small nuclei appear in the body of the organism at this stage (fig. 37), and there is some evidence of a cell multiplication by budding (figs. 38, 44, 51). It is entirely possible, however, that a multiplication of the nuclei in either the large or the small motile bodies may occur without any nuclear fusion. The observations at this point do not justify very definite conclusions, since the staining of the zoospores *in toto* makes the following of the nuclear phenomena decidedly difficult. There seem, however, to be some grounds for maintaining that the phenomena just described constitute a true case of heterogamy. One cell is characterized by a large body, slow movements, and a nucleus of average proportional size. On the other hand, the smaller cells are swift of movement and have nuclei very large in proportion to their size. The oil drop probably serves as reserve food for the temporary nutrition of either gamete in case fusion does not occur, or of the zygote in case it does occur.

In order to secure the development of the zoospores upon the host, young alfalfa seedlings were carefully washed and introduced into small vessels containing sterile distilled water, so that only the roots and the lower parts of the stem were immersed. A gall from an infected plant was carefully washed, crushed, and introduced into the water. After one day the seedlings were removed from the water, crushed under cover slips on slides, and examined for evidence of the presence of the parasite. On and in the tissues of the host which had been at the water level were numerous

amoeboid bodies, as well as zoospores that still retained their characteristic form. The cilia, however, were no longer to be seen. The amoeboid bodies were watched for some hours and were seen to become clustered and to move in masses; they could also be seen to bud at times, much as in the case of the ciliated cell shown in fig. 38. Budding of the amoebae in the infection stage has been reported to occur in *Plasmodiophora* (11). Seedlings left under these conditions developed small galls at the bases of the secondary roots.

To secure stained preparations of the plasmodium in its early stages, young alfalfa seedlings were grown in a pot in which a badly infected mature plant was also growing. At various times seedlings were removed and examined. Small galls were soon found at the bases of the secondary roots of some of the seedlings. As soon as the plants began to produce crown buds, galls appeared upon the crown (fig. 3). These young galls were fixed, imbedded, sectioned, and examined after various staining processes. The plasmodium of the parasite was found to be widely extended in the galls. An amoeboid or plasmodial vegetative condition of the parasite within the host has often been noted in members of the Chytridiaceae. FISCHER (15) and BARRETT (4) have observed it in species of *Olpidiopsis*. CORNU (7) observed it, as well as the amoeboid movement of the zoospores, in various members of the family. He also noted a suggestion of cleavage in what he called the plasmodium of *Rozella*, and suggested its formation by the union of many amoeboid zoospores. FISCHER went so far as to classify several genera upon the basis of differences in the plasmodia. He observed that the protoplasm of the parasite mingled intimately with that of the host and gradually displaced it. FISCH's (14) description of *Reesia amoeboides* is a striking suggestion of the near relationship of the group to the Myxomycetes. A secondary infection of cells occupied by the plasmodium is of common occurrence. As a result, the cells of the galls are often occupied, not only by the plasmodium, but also by the earlier stages of the parasite (figs. 39, 41).

The nuclei in the plasmodium are very numerous, corresponding quite closely in size and appearance to those in the resting spores

(figs. 42, 43, 45). The central clear portion surrounded by peripheral chromatin is characteristic of their appearance, although in many cases the whole nucleus takes up the chromatin stains. The cytoplasm of the plasmodium is slightly granular and stains very lightly. It has not yet been possible to keep the plants infected in the greenhouse long enough to secure the development of the resting spores within the galls.

In order to examine the plasmodia in older galls, sections were stained with the triple stain and with haematoxylin. Only comparatively young galls were sectioned, as considerable foreign mycelium was found to be present in the older galls. Infection by other fungi, and the consequent presence of a foreign mycelium which has no connection with the organism causing the galls, is to be expected because of the contact of the infected parts with the soil. Sections of smooth intact galls showed no such signs of foreign contamination. The same type of plasmodium, however, that was found on the exterior of seedlings exposed to infection, and within the tiny galls formed upon seedlings grown in infected soil, was also found throughout the cells of the galls containing resting spores. In marginal cells of some galls the amoeboid cells were found in numbers (fig 47), preceding the formation of a plasmodium such as is shown in fig. 5.

In some cases the plasmodium forms an irregular streaming mass, forcing its way through and between the cells of the gall tissue (fig. 45). In other cases it ramifies through the cells as a network of naked protoplasm (fig. 42). Multiplication of the plasmodia may occur in these stages by budding and fragmentation (fig. 51). The walls of the host cells are often dissolved or gelatinized in advance of the main body of a plasmodial mass, presumably by enzymatic action (fig. 4).

The resting spores are formed in cavities or pockets occupied by the parasite. Remains of host walls are mingled with the plasmodial masses of the parasite, and a clear staining of the material at this stage is almost unattainable. A yellow coloration pervades the unstained content of the pockets previous to the formation of the spores. At the time of spore formation the yellow coloration is limited to the outer walls of the spores, their content being

clear and refractive. Just how the formation of the spores takes place has not been determined.

Around the margins of the larger cavities containing spores the naked protoplasm of the plasmodium may be found in a more or less shrunken condition. The similarity of this protoplasm to that within the spores is convincing evidence of the origin of the spores from the plasmodium, the most noticeable difference being in the more regular arrangement of the nuclei within the spores. Isolated host cells or groups of cells may be occupied individually by separate plasmodia; it would seem that these small plasmodia may develop walls about themselves and become resting spores. The subject of spore formation is in need of very careful and prolonged investigation.

Primary infection, the actual entrance of the parasite into an uninfected host, has not yet been observed. The fact that the parts invaded are the adventitious buds and the secondary roots in the very earliest stages of development minimizes the chances of such an observation. There is little doubt, however, that either the zoospores before fusion, the zygotes, or the plasmodia formed on the surface of the host may penetrate the embryonic host tissues. In young bud galls cases have been found in which practically every cell of the growing tip was occupied by the parasite (fig. 7). From this figure it will be seen that the infecting cells of the parasite vary greatly in form and size after penetration. Often they are found in contact with the host nucleus, the latter being still intact. Sometimes a host cell contains several of the invading cells. Sometimes a fusion of a number of these invading cells, preparatory to the formation of a larger plasmodium and the breaking down of the separating host cell walls, may be noted. All these features are illustrated in fig. 7.

The discussion of the studies upon the life history of the parasitic organism may be concluded by a brief reference to the subject of nuclear divisions in the different stages. Had division figures not been found, identification of the nuclei in the plasmodium would have been questionable, since these nuclei are very small and details of their structure are not easily distinguished. Division figures, however, are very common in some of the prepara-

tions, and are easily recognized as such upon careful examination. The process of division is clearly mitotic (figs. 46, 48–50, 53–57). Dark bodies suggesting centrosomes are commonly visible in the metaphases at the poles of the spindles (figs. 46, 50). The early divisions of the nuclei in the amoeboid infection stages have been followed with only partial success. The figures correspond to those seen in the later divisions, but average considerably larger (fig. 44). The centrosomes, if the dark bodies at the poles of the spindles are to be called such, are a constant feature in good preparations. Clearly recognizable division figures within the sporange have been found less frequently. There is some evidence that a division occurs while the nuclei are yet very small (fig. 15), and much better evidence of a division just preceding the formation of zoospores (figs. 17, 48). Whether these are in the nature of reduction divisions must be left for future determination. Careful study has failed to lead to a definite conclusion as to the chromosome number. In the divisions immediately preceding the formation of the zoospores the number of chromatic bodies seen is apparently four (fig. 48). In all the division figures observed, at whatever stage, the spindle seems to be intranuclear, corresponding to the findings of other investigators of members of the Chytridiaceae.

Classification of parasite

VON LAGERHEIM (**19, 28**), who first noted the occurrence of the crown-gall of alfalfa, classed it in the genus *Cladochytrium* with the specific name *alfalfae*. MAGNUS (**21**), in his article of 1902, gave strong reasons for removing the organism from that genus and referred it to the genus *Urophlyctis*, retaining the specific name given by VON LAGERHEIM. The terminology *Urophlyctis alfalfae* (von Lagerheim) Magnus has been generally accepted in later works. Although MAGNUS was right in removing the parasite from the genus *Cladochytrium*, it is doubtful whether he was justified in placing it in the genus *Urophlyctis*, on the basis of his limited observations. The description of the genus *Urophlyctis* as given in Saccardo's Sylloge Fungorum (**7**: p. 303) is as follows:

Urophlyctis Schroet. Krypt. Fl. Schles. Pilze. p. 197. (Etym. *oura* cauda et *phlyctis* bulla.) Zoosporangia sessilia in plantis vivis, fasciculis filamentorum

immersa. Sporangia perdurantia intra cellulas plantarum viventium e mycelio filiformi perforatas formata, copulatione cellularum similium orta. Cellulae alterius protoplasma in alteram effunditur, haec vero crescit et membrana crassa cincta in sporangium perdurans mutatur.

The studies of the alfalfa crown-gall organism by MAGNUS did not establish the characters cited by SACCARDO. The descriptions of the genus as given by ENGLER and PRANTL (**12**) and by MIGULA (**24**) also include characters that MAGNUS apparently did not verify.

The writer has failed to observe, in the organism studied, the characters ascribed to the genus *Urophlyctis*. In view of the incompleteness of the results, no change of classification is suggested at the present time. It seems highly desirable, however, that careful investigations be made of the various organisms referred to this genus. Possibly it may prove necessary to discard some of the previously used diagnostic characters and to redescribe the genus on the basis of fuller observations. The relationships of the Chytridiaceae are in an unsettled state. There have been many suggestions in the literature that would lead one to question whether or not this family finds its proper place among the Phycomycetes. The studies upon which classification has been based in many cases have been very superficial, and few efforts have been made to follow out complete life histories. Cases in which an amoeboid or plasmodial stage has been noted, with the absence of anything resembling mycelium excepting naked threads of protoplasm, furnish reason to suspect that the family is more closely related to the Myxomycetes than to the Phycomycetes.

Summary

1. The resting spores, placed in water cultures, develop into sporanges.

2. Within these sporanges are formed motile zoospores of two sizes; frequently one large zoospore and many small ones are formed in the same sporange.

3. One or several small zoospores may become attached to one large zoospore. Only one remains permanently attached. There is some evidence that this attachment is related to a sexual fusion.

4. The movement of the large zoospore continues after the attachment of the small one.

5. The small zoospores, the large zoospores, and the united zoospores (zygotes?) become amoeboid after a period of motility.

6. In the amoeboid state, singly or in groups, these bodies may be observed upon the surface of the host.

7. In infected soil young alfalfa seedlings develop galls in which plasmodia are found.

8. In older galls similar plasmodia are found, which ramify through the tissues of the gall.

9. The resting spores are formed in cavities within the tissues of the galls.

10. The cytoplasmic and nuclear contents of the resting spores in the dormant condition correspond to those of the plasmodium in the stage immediately preceding the formation of resting spores.

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EXPLANATION OF PLATES VII–X

With the exception of plate VII, the figures were drawn with the aid of an Abbé camera lucida at table level. Leitz oculars and objectives were used, giving the magnifications indicated.

PLATE VII

FIG. 1.—Alfalfa plant badly infected with crown-gall; $\times\frac{1}{2}$.

FIG. 2.—Single galls; $\times 1$.

FIG. 3.—Seedling infected in greenhouse, galls beginning to develop at crown; $\times 1$.

FIG. 4.—Section showing action of parasite upon walls of host cells in advanced stage; parenchyma tissue; $\times 300$.

FIG. 5.—Plasmodium on border of gall; $\times 300$.

FIG. 6.—Sections of resting spores showing outer and inner layers of wall; $\times 130$.

FIG. 7.—Section of young gall on seedling showing cells of parasite in host cells; some of former fused into tiny plasmodia, others in contact with host nuclei; $\times 300$.

FIG. 8.—Section of gall showing cavities and resting spores of parasite.

PLATE VIII

FIG. 9.—Unstained resting spore, view of hollowed surface; $\times 750$.

FIG. 10.—Unstained resting spore, side view; $\times 750$.

FIG. 11.—Resting spore conforming to host cell in which it has developed; $\times 750$.

FIG. 12.—Unstained resting spore showing marginal vacuoles; $\times 750$.

FIG. 13.—Irregular relation of walls of resting spore; $\times 1800$.

FIG. 14.—Unstained sporange when first put into hanging-drop culture; $\times 750$.

FIG. 15.—Section of resting spore; may be stage just following resting period; $\times 1800$.

FIG. 16.—Group of nuclei in sporange; chromatin in marginal aggregations; $\times 1800$.

FIG. 17.—Division figures in sporange, apparently just preceding zoospore formation; large nucleus destined to be nucleus of large zoospore; $\times 1800$.

FIG. 18.—Spore formation about completed, unstained; note rupture of outer wall; $\times 750$.

FIG. 19.—Unstained sporange containing a few zoospores; most of those formed already escaped through prominent opening; $\times 750$.

FIG. 20.—Section of sporange just preceding formation of zoospores; $\times 1800$.

FIG. 21.—Section of sporange showing large nucleus which will be included in large zoospore; $\times 1800$.

FIG. 22.—Marginal portion of sporange showing beginning of cleavage into zoospores; $\times 1800$.

FIG. 23.—Zoospores almost fully formed in sporange; $\times 1800$.

FIG. 24.—Nuclei of sporange showing nucleoles; $\times 1800$.

FIG. 25.—Zoospore just before exit from sporange; $\times 1800$.

FIG. 26.—Nuclei of resting condition of spore.

FIG. 27.—Section of sporange dividing into zoospores; note characteristic arrangement of chromatin in knot at one end or side of nucleus; ×1800.

PLATE IX

FIG. 28.—Free zoospores of two sizes; ×1800.

FIG. 29.—Group of large zoospores formed in same sporange; ×1800.

FIG. 30.—Large and small zoospore within same sporange; ×1800.

FIGS. 31, 32.—Two large zoospores; ×1800.

FIG. 33.—Large and small zoospore showing projection of former toward latter; position may be accidental; ×1800.

FIGS. 34, 36.—Attachment of small zoospores to large; ×1800.

FIG. 35.—Binucleate zygospore following fusion(?); ×1800.

FIG. 37.—Amoeboidal stage following fusion, or perhaps developing without fusion; nuclei have multiplied; ×1800.

FIG. 38.—Apparent budding in amoeboid stage; ×1800.

FIGS. 39, 41.—Plasmodium and young infecting amoebulae in same host cells; ×1800.

FIG. 40.—Free zoospore, small size; ×1800.

PLATE X

FIG. 42.—Plasmodium spreading through tissue of host; host nucleus visible; ×1800.

FIG. 43.—Plasmodium breaking through wall of host cell; ×1800.

FIG. 44.—Amoebulae within tissues of host; note nuclei in division; ×1800.

FIG. 45.—Note as for fig. 42; no host nucleus visible.

FIG. 46.—Nuclei of plasmodium in division; ×1800.

FIG. 47.—Amoebulae massed in marginal cells of gall; ×1800.

FIG. 48.—Division figures in sporange; ×1800.

FIG. 49.—Prochromosomes(?) in nuclei of sporange; ×1800.

FIGS. 50, 53.—Anaphases in plasmodium; ×1800.

FIG. 51.—Budding or fragmentation of plasmodium; ×1800.

FIG. 52.—Nuclei of sporange just preceding formation of spores; ×1800.

FIGS. 54–57.—Division figures in plasmodium within host; ×1800.

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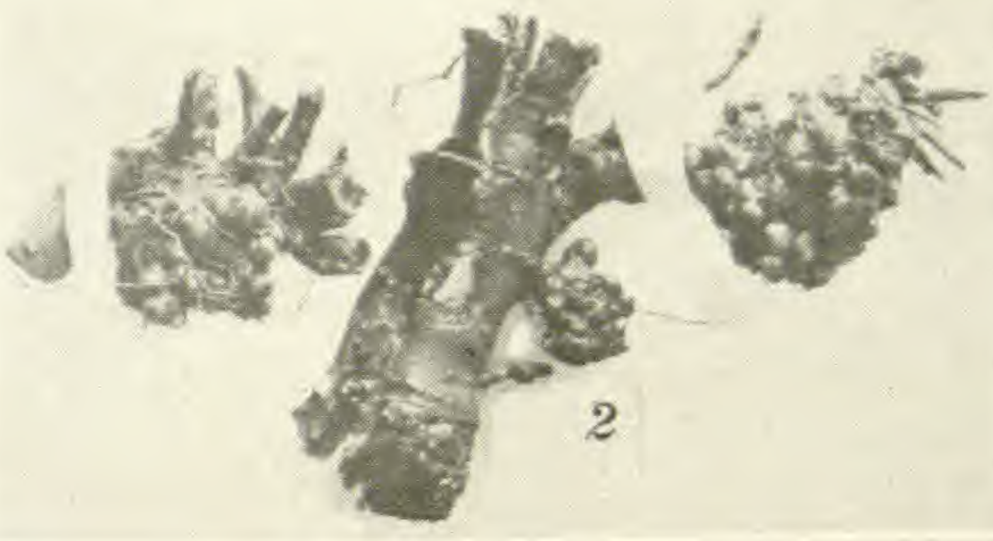
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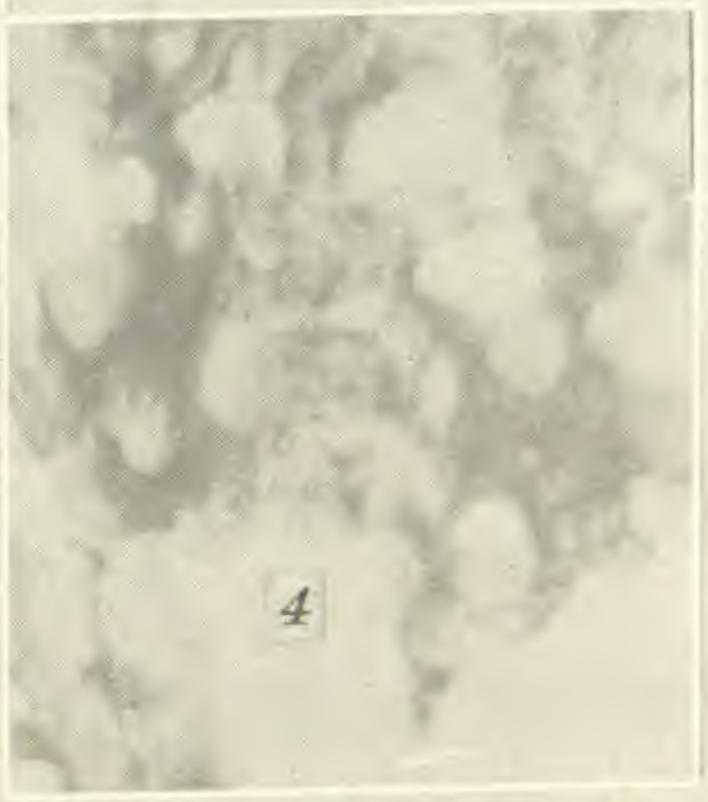
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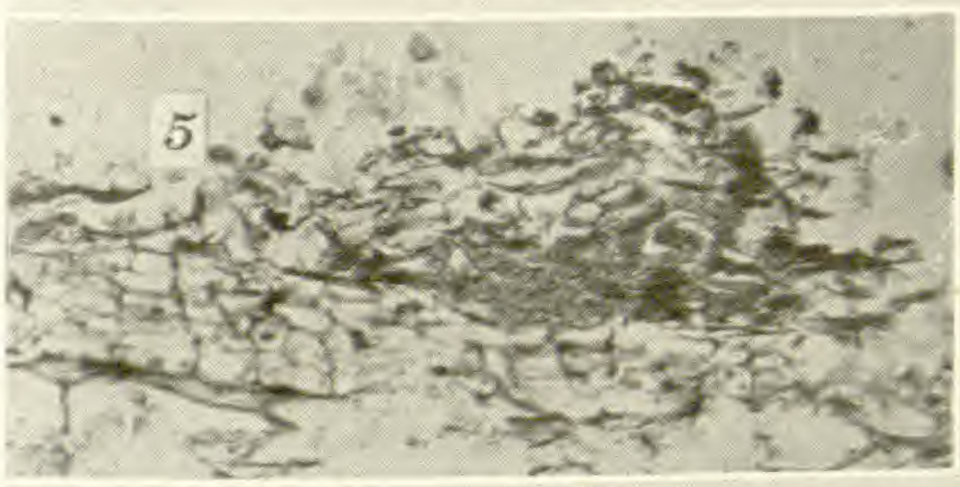
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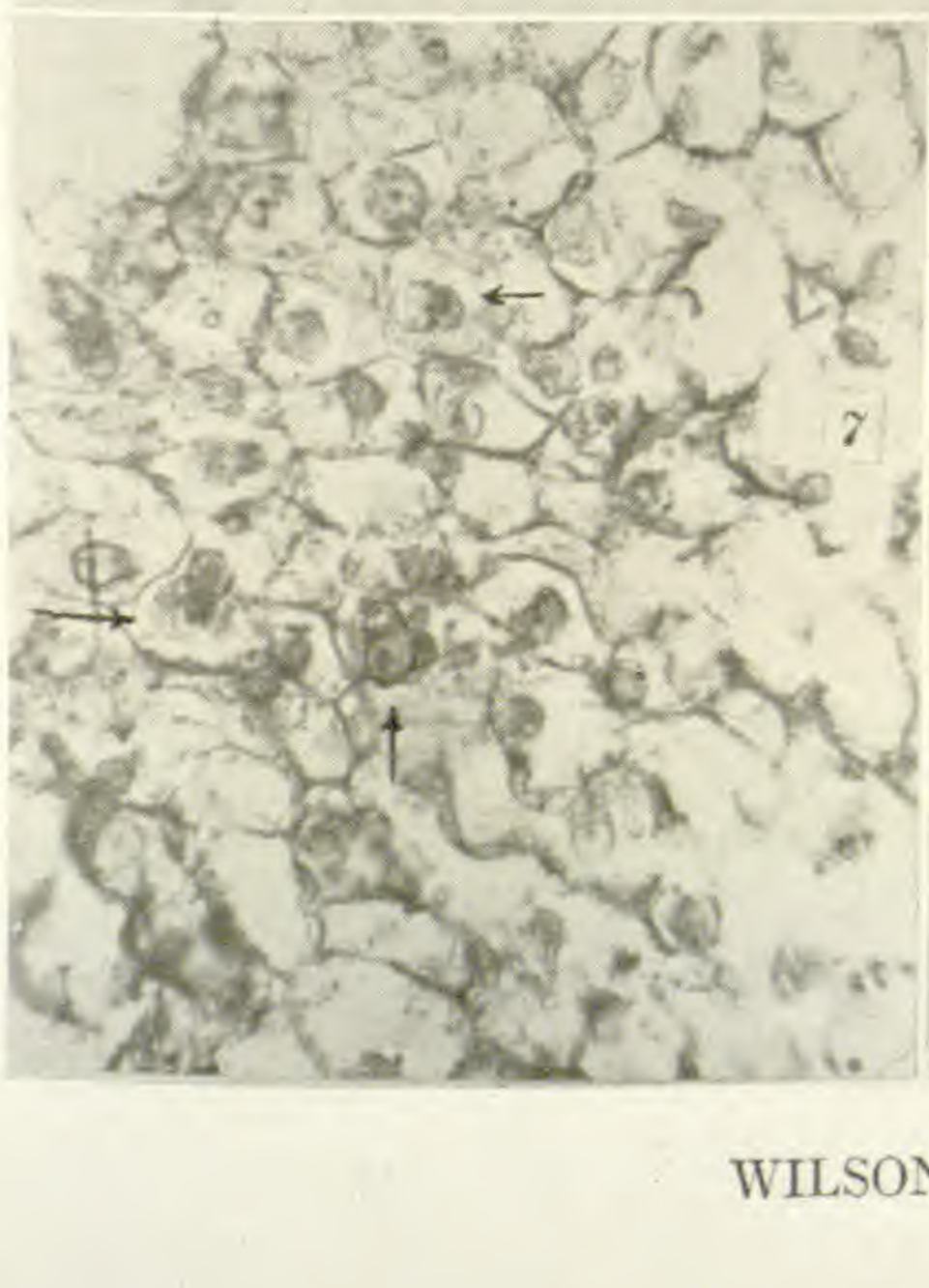
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WILSON on CROWN-GALL

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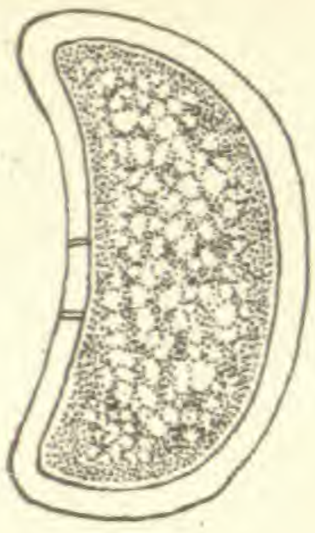

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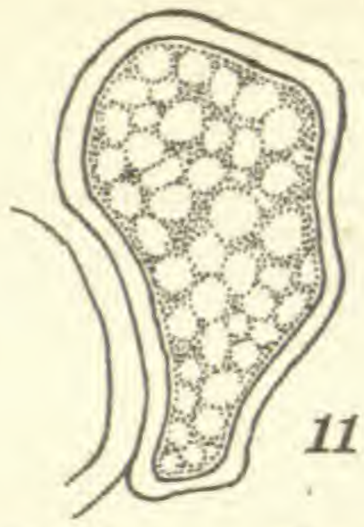

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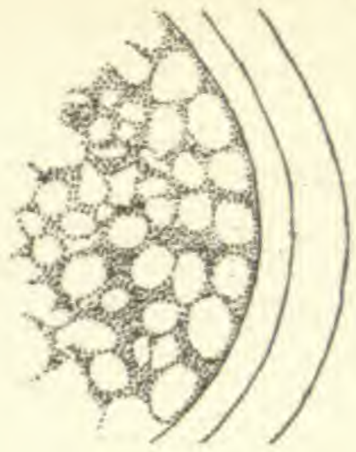

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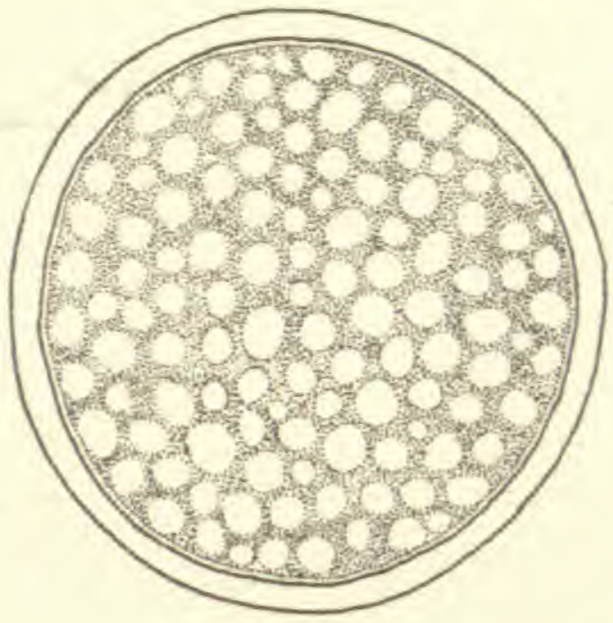

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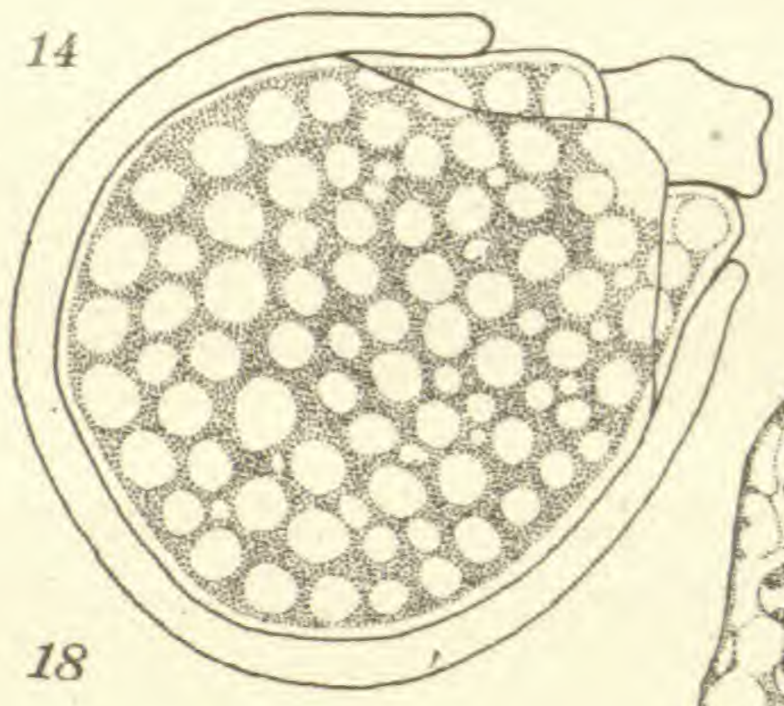

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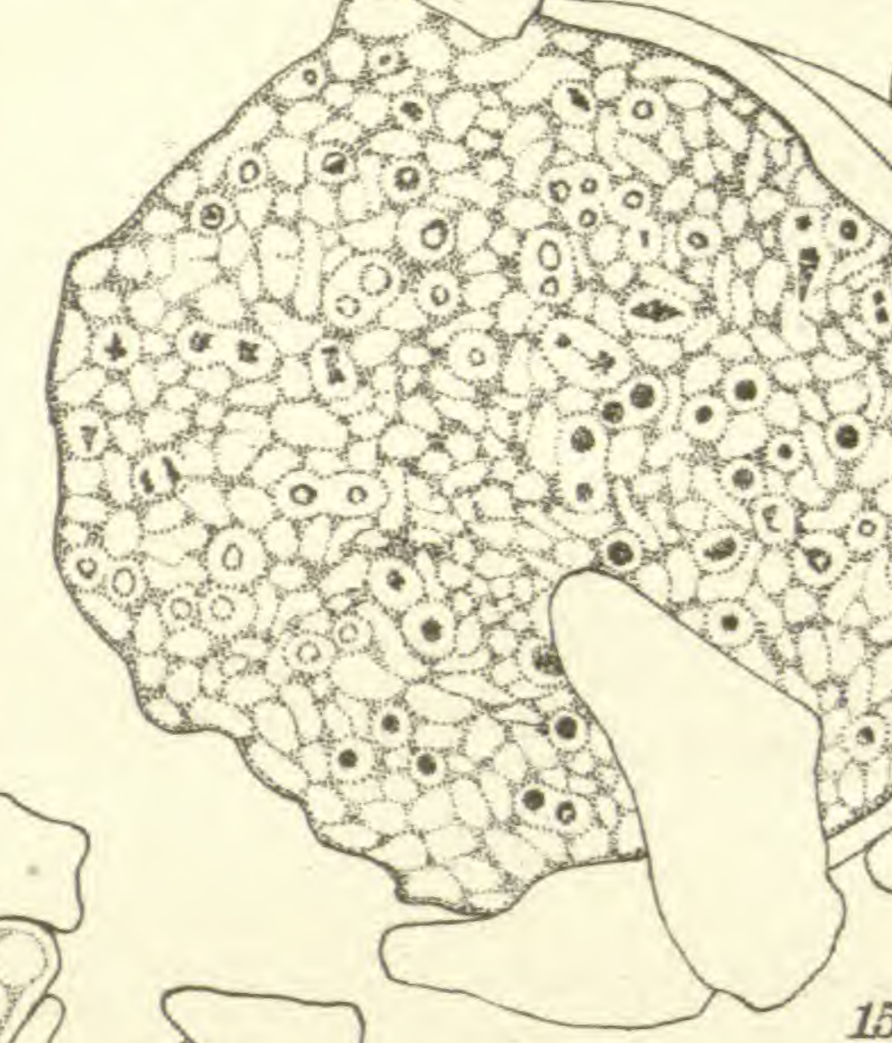

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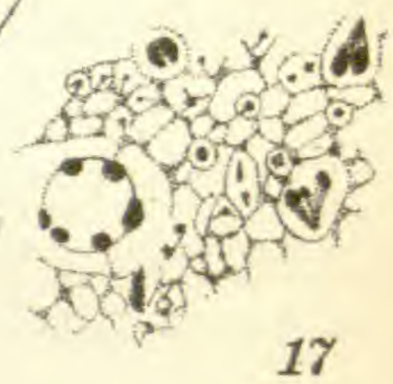

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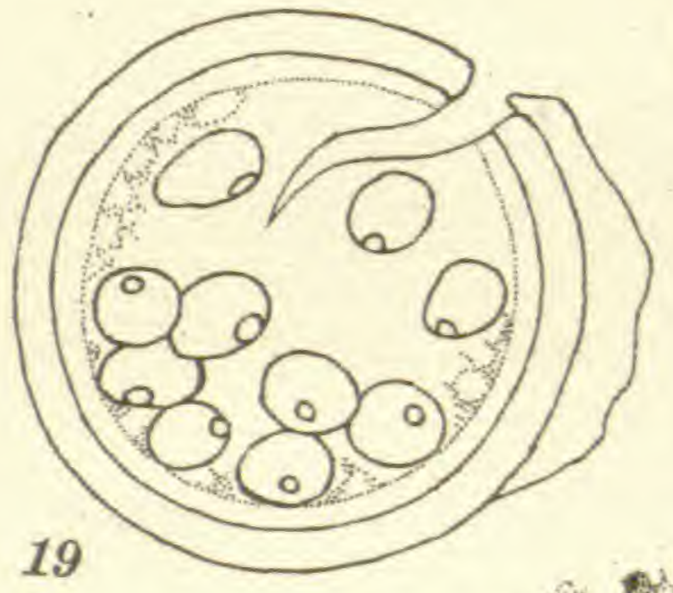

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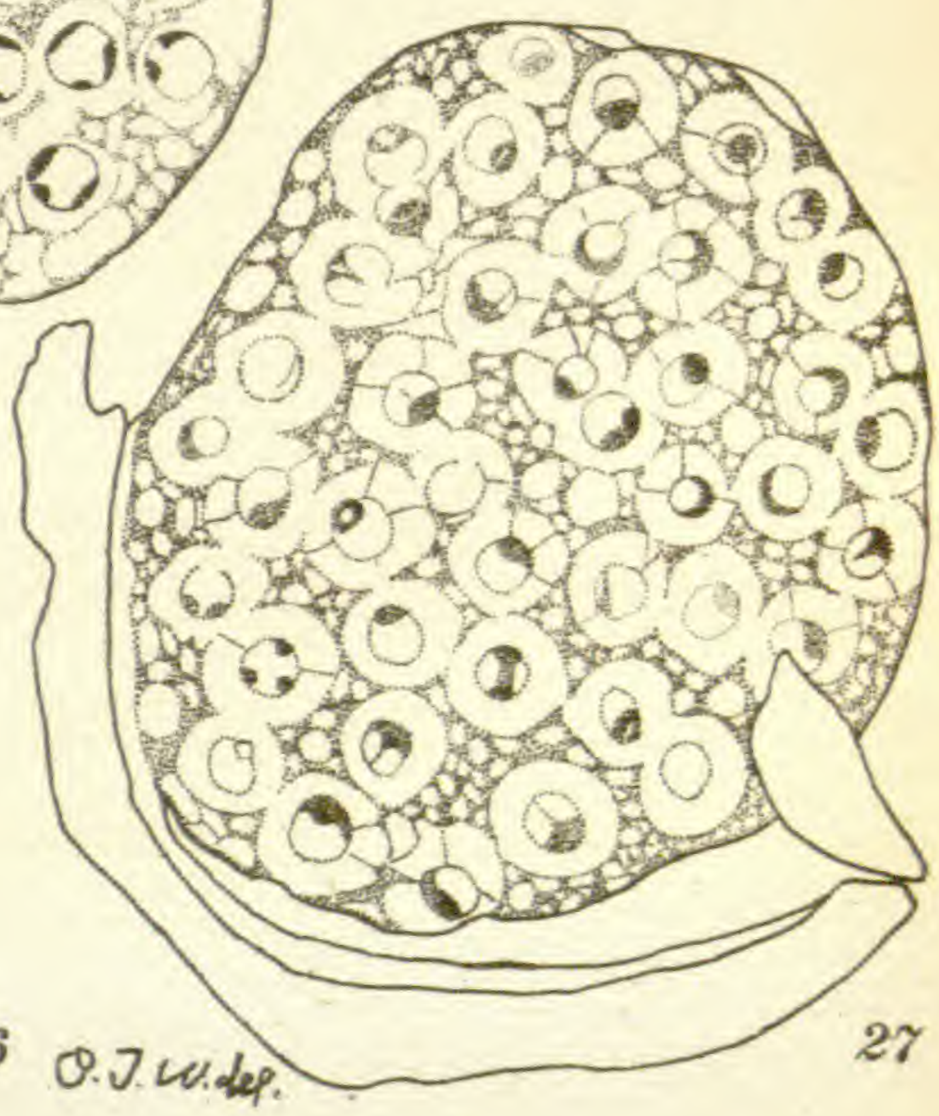

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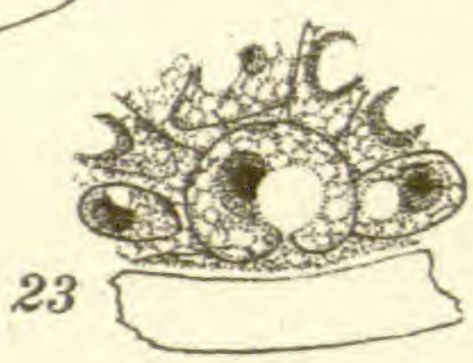

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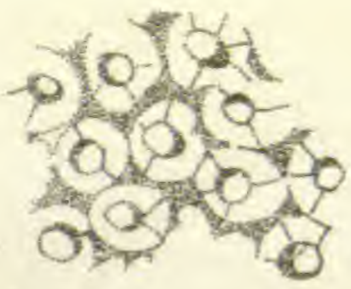

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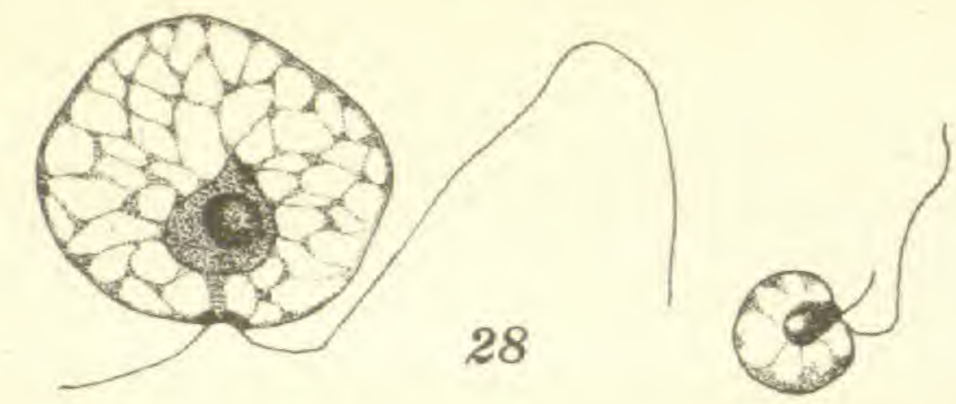

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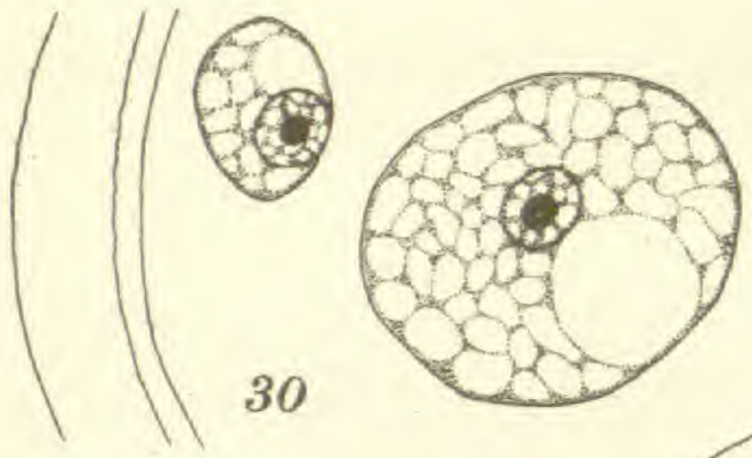

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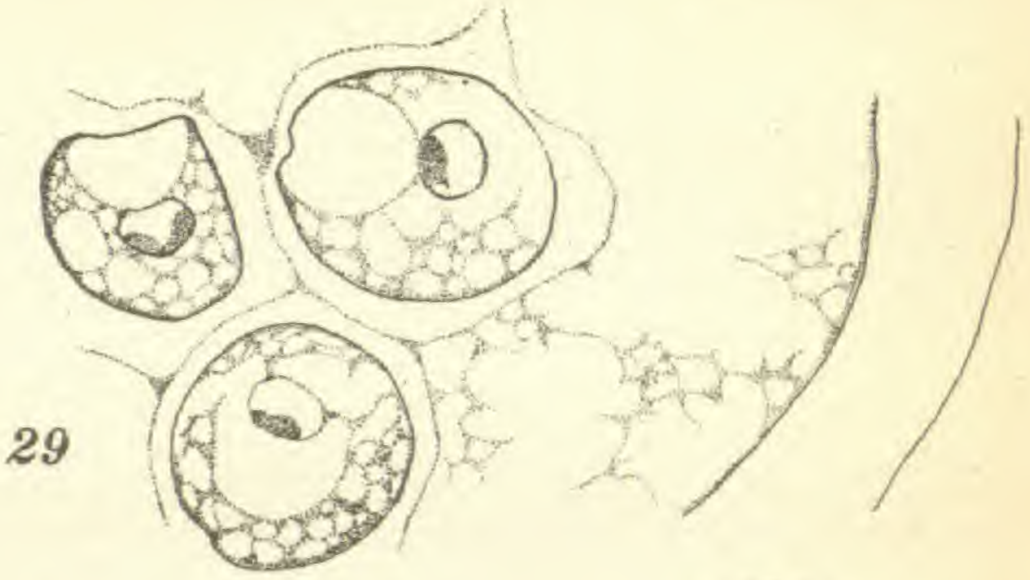

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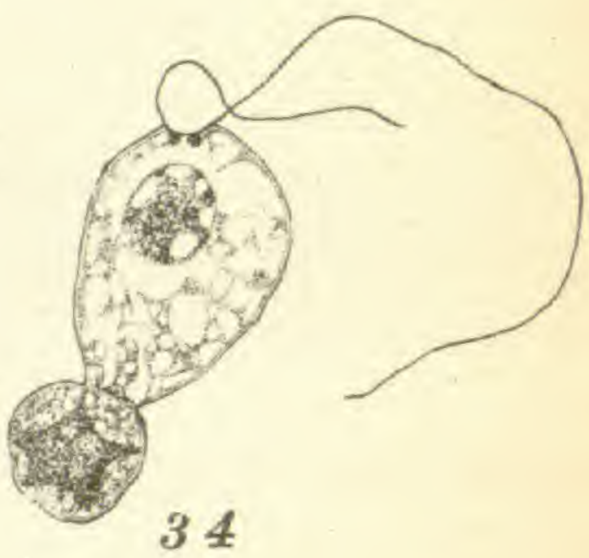

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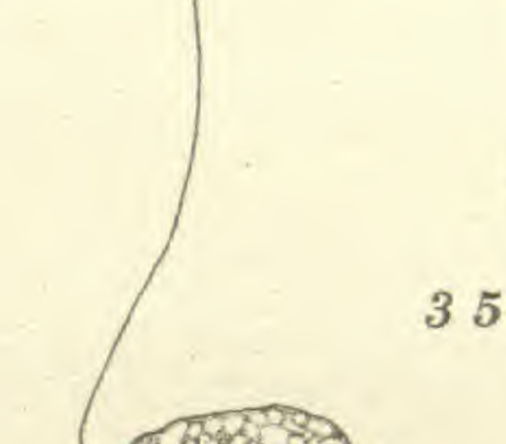

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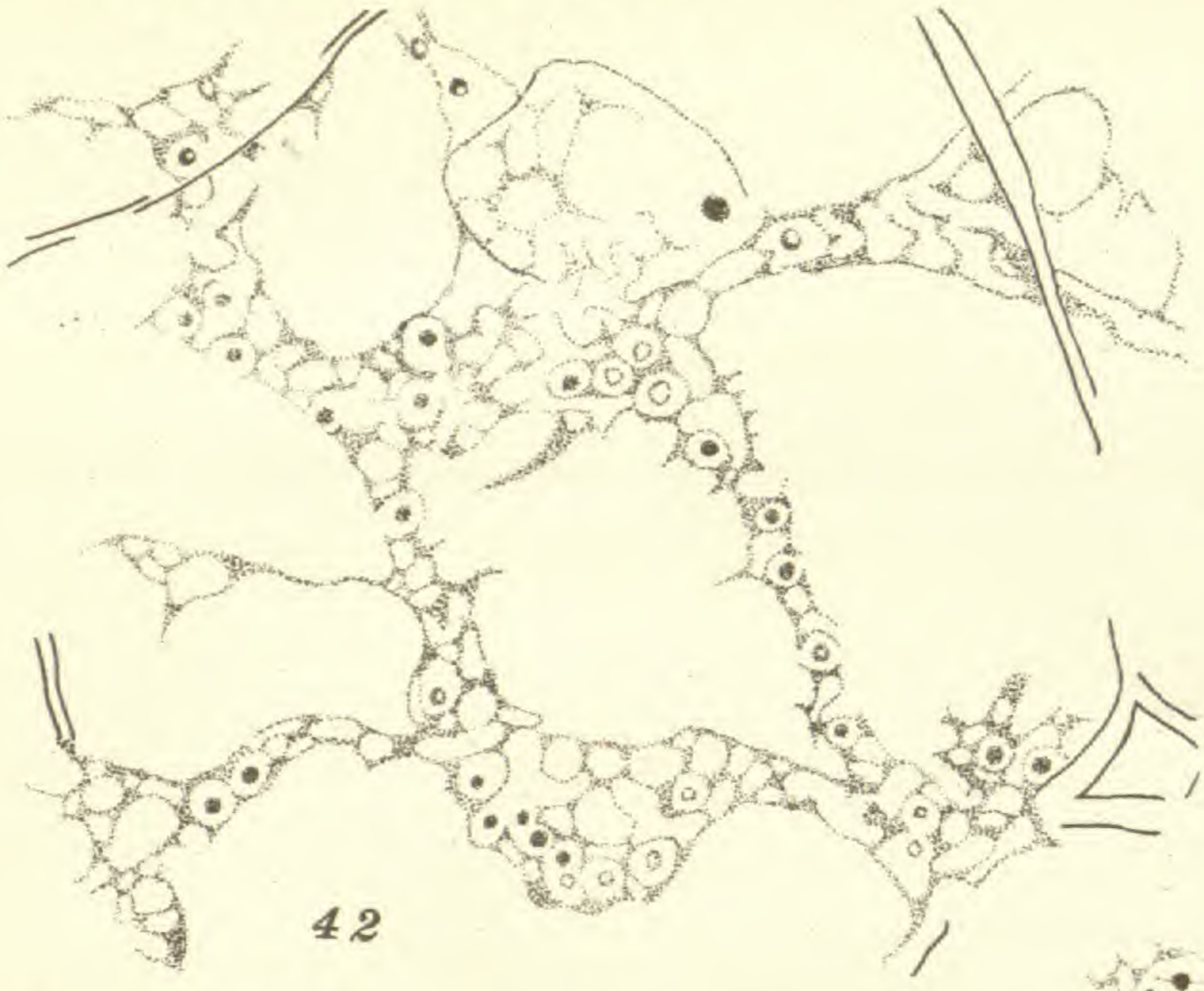
WILSON on CROWN-GALL



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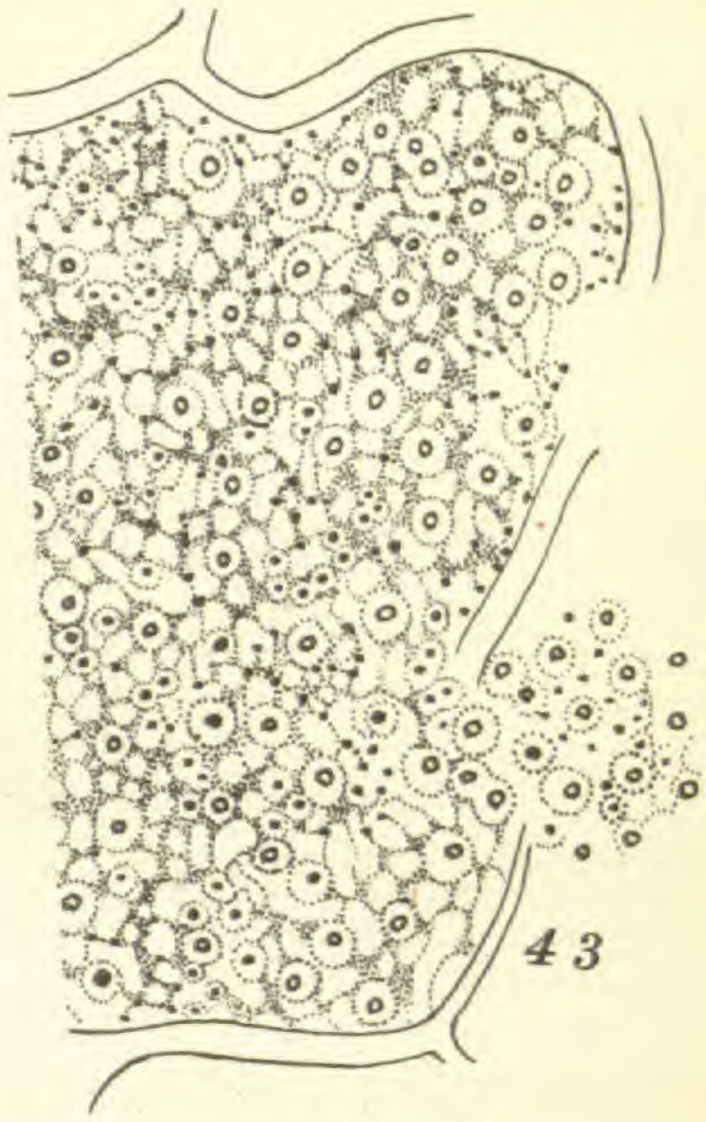

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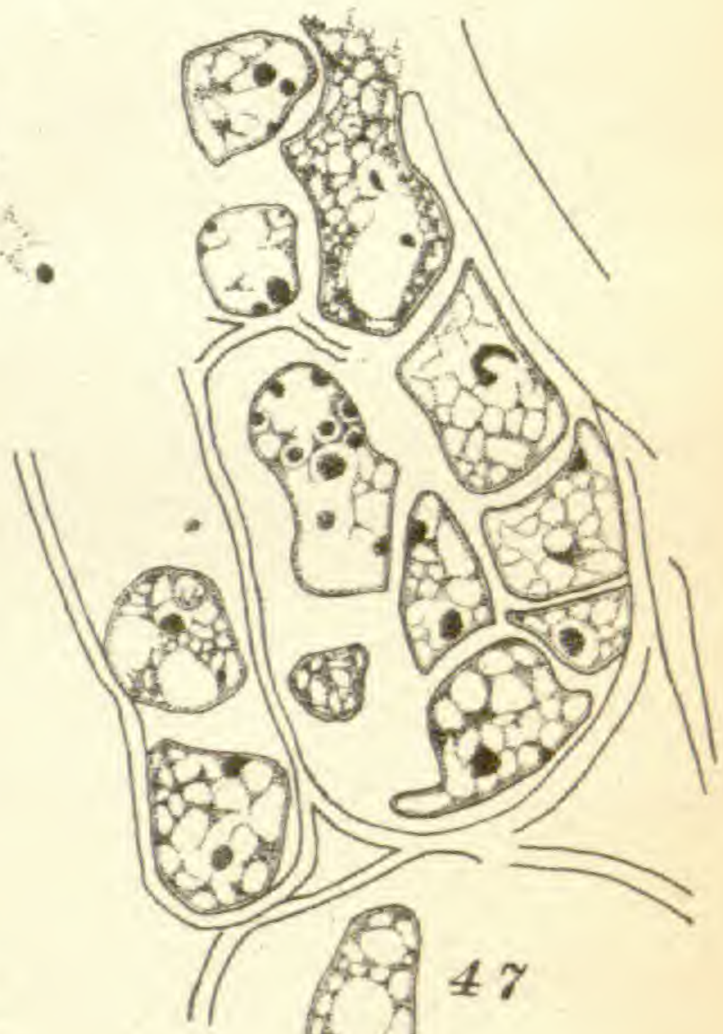

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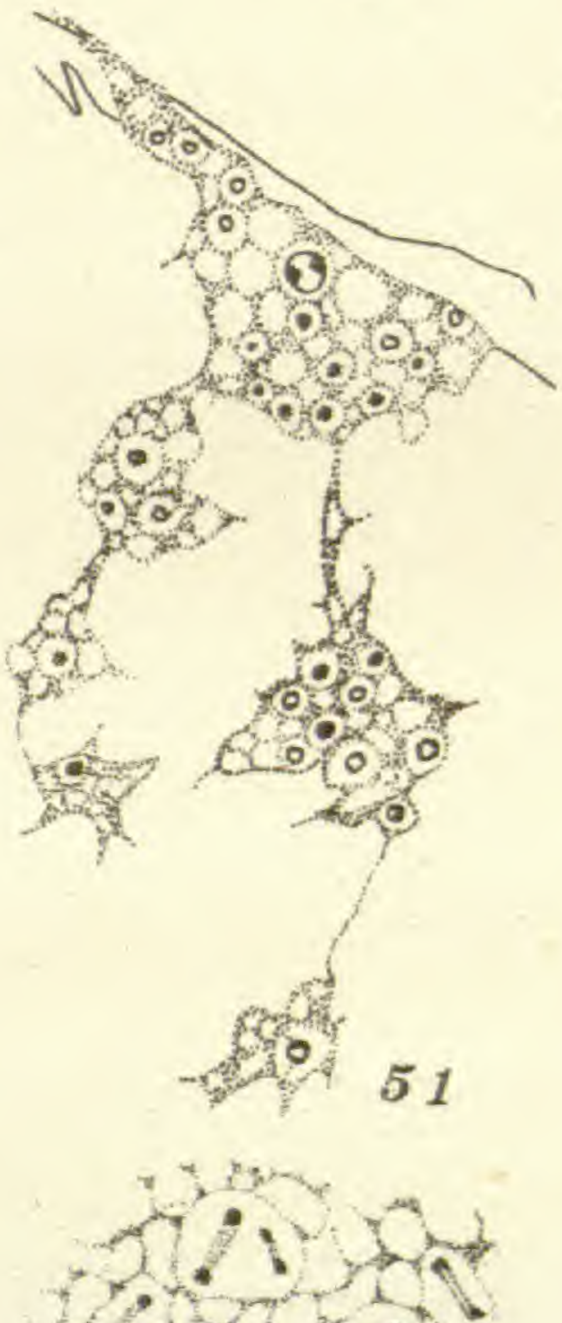

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WILSON on CROWN-GALL

GEO-PRESENTATION AND GEO-REACTION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 268

EVA O. SCHLEY

(WITH FIVE FIGURES)

Historical

The reaction of plants to geotropic stimulation has been the subject of considerable investigation, the problem having been attacked from many standpoints. Naturally, perhaps, the physical side was studied first, a number of workers having developed the main features of gravity stimulus, presentation and reaction times, perception, conduction and response, organs of perception, and other related subjects. The chemical side of the field, involving the change in metabolism of the stimulated organ, has received much less attention.

The first worker in this field seems to have been KRAUS. As early as 1870 he published (14) the first of a series of researches on the chemical content of the growing plant, both in normal relations and after subjection to various external stimuli. This research included (1) the water content, (2) the acidity, (3) the sugar content of the normally growing shoot, (4) the relation of each to the growth maximum, and (5) steps in the change of the cell content of the concave and convex side of the geotropically and heliotropically responding organ. He determined that, in the normally growing shoot, (1) the acidity decreases from the tip downward, (2) the water increases relatively from the tip to the downward limit of growth, and (3) the sugar increases from the tip below the growth maximum and therefore is not a limiting factor in growth. In the stimulated organ he found on the convex-becoming side (1) an increase of sugar production up to the time of visible curvature and then a decrease, (2) a progressive decrease in acidity during stimulation, free acid being entirely absent from the responded organ, and (3) a progressive increase of water preceding curvature.

DeVries (5) studied the forces released in gravity stimulus, the effect of these released forces upon curvature, and the release of elasticity by gravity stimulus. He concludes that gravity produces an increase of osmotically active material in the cells of the convex-becoming side, causing an intake of water from the adjacent tissue, the resulting increased turgor producing a longitudinal extension of the elastic cell membranes, which, originally plastic, become fixed through growth and lignification.

Cielsielski (2) observed a difference of the cell sap on opposite sides of geotropically stimulated roots, the cells of the convex-becoming flank exhibiting a thin watery protoplasm in contrast with the denser, more opaque plasma of the side becoming concave. Kohl (13) obtained analogous results in the sporangiophores of *Phycomyces*, in that in geotropic stimulation the plasma of the concave side of the filament became much thicker, while that of the convex side became thin and watery. He concluded that there was a causal relation between this differentiation of cell plasma and the curvature of the organ. Elfving (7), however, according to his reviewers, produced a similar differentiation of protoplasm in *Phycomyces* sporangiophores by allowing them to push against a glass obstruction, a purely mechanical stimulation.

Hilberg (11), contrary to DeVries' results, found that in geotropic stimulation the osmotic pressure of the concave side of leaf joints and stem nodes of various plants is greater than that of the convex side. Wortman (22) negatives both DeVries' and Hilberg's conclusions, since he could find no difference in the osmotic pressure of the two flanks of stimulated organs, and holds DeVries' view of the causal relation between turgor and curvature to be wholly untenable. On the other hand, he agrees with Kohl in that he found in geotropically stimulated organs the plasma "wandered" from the convex to the concave side, the thickened plasma inducing the cell membranes of the concave side to become thicker but less elastic and less extensible than those of the convex side. These latter, stretching longitudinally, force the concave side upward, thus producing curvature. Noll (16) confirms Wortman's work, but refutes his argument of the causal relation between the changed activity of the plasma and the curvature of the organ.

COPELAND (3) was unable to detect a difference in the opposite flanks of stems split lengthwise and stimulated geotropically four days. KERSTAN (12), using the plasmolysis method, found no increase of turgor in either flank of geotropically or heliotropically stimulated shoots either during curvature or after its completion. He concludes with NOLL that the decrease of turgor is due to the fact that the osmotic producing substances do not keep pace with the intake of water of the cells and their increased volume.

THATE (20) found KRAUS'S method too crude to determine the difference of water in the two flanks of heliotropically stimulated shoots, although he does not dispute its existence. On the other hand, TONDERA (21) was able to verify KRAUS on this point and from his studies developed the law: "As the cells of the rind parenchyma of the lower organ half become filled by the streaming of water, due to gravity, the cells of the opposite half become water-poor, the resulting difference in pressure forcing the organ to move toward the water-poor half." This at best is a very crude conception.

From the cytological standpoint, MCDOUGAL (15) found that the cells of the convex side are greater in length, breadth, and thickness than those of the corresponding tissue of the concave side of geotropically stimulated roots. GEORGEVITCH (8) confirms this earlier work, while BÜCHNER (1) found the same condition in shoots that had been prevented from responding to gravity stimulation.

CZAPEK (4) is probably the chief worker in the chemical field of geo-presentation and reaction. Working with normal seedlings, he found that homogentisic acid is produced as a product of the oxidation of tyrosin, through the action of an oxidase, tyrosinase. In geotropic stimulation the tyrosin is converted into homogentisic acid by tyrosinase, as in normal seedlings, but the further oxidation of the homogentisic acid by the oxidase is inhibited by the production of an anti-oxidase, which renders the oxidase partly ineffective and by this means causes an accumulation of homogentisic acid. The accumulation begins after five minutes' stimulation, reaches a maximum at the time of distinct curvature, and disappears when reaction is complete. GROTTIAN (10) and GRAFE and LINSBAUER (9), however, were unable to confirm his results. They found as

great variation in the amount of homogentisic acid in different analyses of normal shoots as CZAPEK found between the stimulated and unstimulated organs.

In her work on thermotropism of roots, ECKERSON (6) found the greater permeability to be on the concave side of the root, and that this permeability changed with the changed thermotropic reaction of the root.

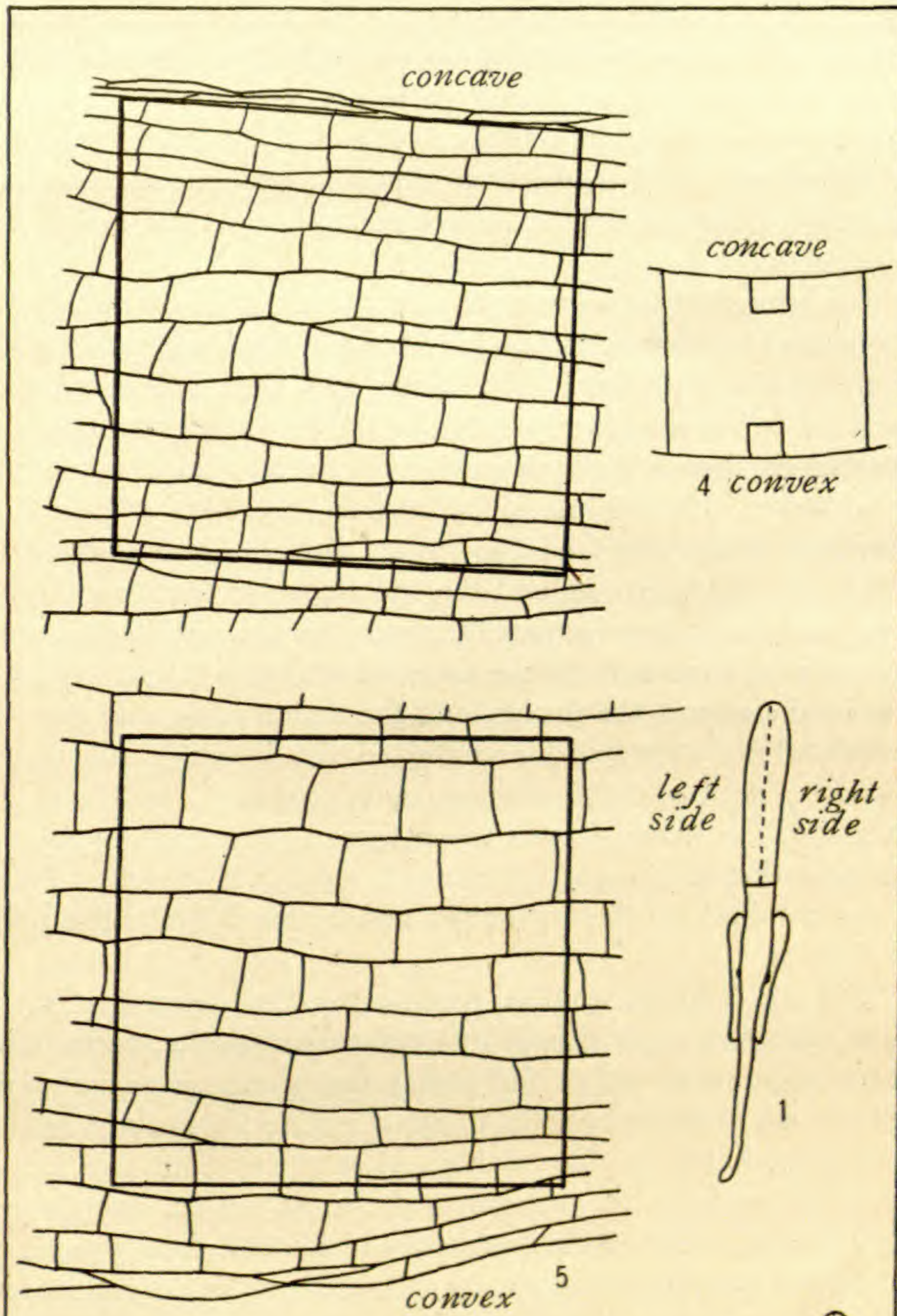
Scope of experiment

The work here presented is a continuation of that reported in a previous paper (18), which dealt with the acidity of the normal shoot, and compared the acidity of the two flanks of the geotropically stimulated shoot. The present paper deals with the changes in metabolism of the carbohydrates, the difference in osmotic pressure, and the difference in respiration of the upper and lower flanks of the geotropically stimulated shoot through presentation and reaction periods. *Vicia Faba* seedlings were employed throughout the experiment because they respond readily to geotropic stimulation, and because they are large enough to be easily split longitudinally. Trouble was experienced in germinating the seeds because of the development of mold. To overcome the difficulty the seeds were washed in tap water and then soaked two or three minutes in a 1 per cent solution of silver nitrate and rinsed thoroughly. They were grown in sand which had been sterilized by boiling in water an hour or longer, and then put into sterile pots while hot and allowed to stand until the following day before planting the seeds.

Carbohydrates and proteins

For this analysis etiolated seedlings of *Vicia Faba*, grown in sand in the greenhouse at a temperature of about 20° C., were used. When the seedlings were 6–8 cm. high they were geotropically stimulated for periods ranging from 15 minutes to 5 hours. Duplicate analyses of samples for each period of stimulation, as well as duplicate analyses of the unstimulated organ as controls, were made.

The epicotyls were split longitudinally into right and left halves (fig. 1) in the controls, and into upper and lower halves


concave
concave
4 convex
left side
right side
1
5
convex
upper
2 lower
concave
3 convex

FIGS. 1–5

(fig. 2) in the stimulated seedlings. These latter become the concave and convex halves (fig. 3) respectively in the responded organ. Samples varying from 6 to 10 gm. were used. The fresh portions were weighed in weighing bottles and the weight obtained by difference. The tissue, thus obtained, was cut up fine and triturated, killed in boiling 85 per cent alcohol, and boiled for 30 minutes.

The triturated tissue was subjected to alcoholic extraction for 3 hours and to ether extraction for 2 hours in the Koch modification of the Soxlet extractor. The tissue was then pulverized and extracted in boiling water 30 minutes. This water extraction was repeated six times. Following this was another alcohol extraction of 24 hours. The original killing alcohol, the ether extract (the ether was evaporated and the extract brought into solution in water), the water extract, and the two alcohol extracts were combined, and the volume increased to 500 cc. by the addition of water. This extract contained all the material soluble in these solvents, that is, the sugars, the lipoids, and the amino acids, and may be designated F_1. The residue contained the insoluble substances (starches, pectins, hemicelluloses, and cellulose), and may be designated F_2. From F_1 was taken three 150 cc. portions for the determination of (1) sugar, (2) nitrogen, and (3) dry weight. From F_2 was obtained (1) the dry weight and (2) the hydrolyzable polysaccharides.

The alcohol-water-soluble portion for the determination of sugar was freed from alcohol by evaporation on the steam bath, water being added before and during the process of evaporation, and the final volume brought to 150 cc. The tannins and lipoids were precipitated by the addition of a 10 per cent solution of basic lead acetate, the volume made up to 200 cc. and filtered immediately. The excess lead was precipitated from 150 cc. of the filtered solution by means of a saturated solution of ammonium sulphate, and the volume brought again to 200 cc. Duplicate determinations of 50 cc. portions were made of sugar solutions thus obtained, by the Munson and Walker method for the determination of reducing sugars (17). The amount of cuprous oxide thus obtained was determined by the volumetric potassium permanganate

method (*op. cit.*, pp. 52, 53.). An N/20 solution of permanganate was used. A third 50 cc. portion of this clarified solution was used for the determination of non-reducing or hydrolyzable sugars. The hydrolysis was performed according to the method for the determination of sucrose in the absence of raffinose (*op. cit.*, pp. 40, 41). The cooled solution was neutralized with 20 per cent sodium hydrate, brought to 100 cc. volume, and duplicate sugar estimations made of 50 cc. portions as described.

The residue (F_2), after the determination of the dry weight (to be described later), was used for the determination of the polysaccharides according to the method for direct acid hydrolysis of starch (*op. cit.*, p. 53). Duplicate determinations of 50 cc. portions were used for the determination of sugars, as in F_1.

The calculations were based on the milligrams of copper oxidized in the change from cuprous to cupric oxide, and expressed in equivalent milligrams of dextrose obtained from the Munson and Walker table accompanying the method of analysis (p. 243).

The portion of F_1 for the determination of dry weight was evaporated to moist dryness on the steam bath and brought to constant weight in vacuo. The dry weight of F_2 was obtained by bringing the residue of the original tissue to constant weight in the electric oven at a temperature of 104° C. The calculations for both were based on the dry weight per gram of the fresh material.

The third portion of F_1 was used for the determination of the total nitrogen. This determination was made after the Kjeldahl method as modified by ARNOLD. Calculations were made on the amount of nitrogen per gram of fresh weight. Table I shows the results obtained. It will be noticed that the soluble sugars vary but little throughout. The hydrolyzable sugars increase markedly at the time of visible response, and are greater on the convex side. The polysaccharides decrease as the hydrolyzable sugars increase. The dry weight of F_1 remains practically constant. The dry weight of F_2 remains practically constant until the beginning of curvature, when the weight of the convex side becomes less. The results of this sugar determination are not comparable with the work of KRAUS, for he was working with the raw pressed sap, which probably contained reducing substances other than sugars, as he himself suggests.

KRAUS found that the reducing substances increased on the convex side of the responding organ up to the time of visible curvature, and then decreased on that side of the curved shoot, a point upon

TABLE I

DETERMINATION OF MATERIAL PER GRAM FRESH WEIGHT

TIME STIMULATED	F_3 Soluble sugar (mg.)	F_1 Hydrolyzable sugar (mg.)	F_2 Polysaccharide (mg.)	F_1 Total carbohydrates (mg.)	F_1 Dry weight (g.)	F_2 Dry weight (g.)	F_1 Total nitrogen (g.)
Sample I, unstimulated							
8.74 (right)	23.45	3.21	3.60	30.26	0.06845	0.01920	0.004800
9.35 (left)	23.30	3.95	3.31	30.56	0.06878	0.01876	0.004535
Sample II, stimulated 15 min.							
11.88 (upper)	23.58	2.96	5.75	32.29	0.07124	0.02449	Lost
12.05 (lower)	22.20	3.10	4.93	30.23	0.06894	0.02176	Lost
Sample III, stimulated 30 min.							
10.38 (upper)	19.45	3.54	3.37	26.36	0.06460	0.01809	Lost
10.38 (lower)	19.35	3.58	3.02	25.95	0.06888	0.01887	Lost
Sample IV, stimulated 1 hour							
11.58 (upper)	22.44	4.94	4.00	31.38	0.06296	0.01994	0.004346
12.20 (lower)	22.00	4.73	2.98	29.71	0.06621	0.02014	0.004353
Sample V, stimulated 1.5 hours							
7.17 (upper)	21.90	6.34	4.72	32.96	0.05788	0.02224	0.004238
7.09 (lower)	21.62	4.82	4.66	31.10	0.06379	0.02146	0.004448
	Beginning visible response						
Sample VI, stimulated 2 hours							
10.23 (upper)	18.65	6.20	2.44	27.29	0.05803	0.01828	0.003697
10.15 (lower)	21.50	9.15	3.66	34.31	0.06318	0.01832	0.003838
Sample VII, stimulated 3 hours							
9.32 (upper)	23.10	8.34	3.29	34.73	0.06716	0.02054	0.004421
9.05 (lower)	28.25	9.10	3.50	40.85	0.06802	0.01950	0.004738
Sample VIII, stimulated 4 hours							
9.69 (upper)	23.05	4.90	3.44	31.39	0.07426	0.02100	0.003985
9.66 (lower)	23.45	7.86	3.18	34.49	Lost	0.01875	0.004144
Sample IX, stimulated 5 hours							
6.92 (upper)	21.10	9.20	1.88	32.18	0.06713	0.01907	0.004855
7.63 (lower)	26.75	13.82	2.62	43.19	0.06783	0.01686	0.004593

which he has frequently been misquoted (**14**, pp. 87 and 89). The total nitrogen remains constant throughout, the unstimulated sample I showing almost identically the value of sample IX at the close of the experiment.

Osmotic pressure

The osmotic pressure of the two flanks during the period of presentation and response was determined by means of plasmolysis. Weight molecular solutions of cane sugar and potassium nitrate were used as plasmolyzing agents. The seedlings were geotropically stimulated for varying periods of time. Portions of the seedlings, including the region of response, were sectioned (on the hand microtome) vertically, that is, from upper to lower side of the horizontally placed shoot. The sections were placed in weight molecular solutions of the plasmolyzing agent of such percentages as previous experiment had shown to be close to the plasmolyzing point. The series of weight molecular solutions was graduated to intervals of one-half of 1 per cent. The accompanying tables and graphs show the results of one each of the experiments made.

PLASMOLYSIS

(Using cane sugar as plasmolyzing agent)

In normal shoots both sides plasmolyze at 42 per cent weight molecular
After 5 minutes' stimulation both sides plasmolyze at 42 per cent
After 10 minutes' stimulation both sides plasmolyze at 43 per cent
After 45 minutes' stimulation upper side at 43, lower side at 44 per cent
After 1.5 hours' stimulation upper side at 43, lower side at 44.5 per cent
After 5 hours' stimulation both sides at 43 per cent

(Using potassium nitrate as plasmolyzing agent)

In normal shoots both sides plasmolyze at 31 per cent weight molecular
Stimulated 15 minutes both sides plasmolyze at 31 per cent
Stimulated 30 minutes both sides plasmolyze at 32 per cent, upper general, lower a few cells
Stimulated 45 minutes upper faintly at 33, lower at 33.5 per cent
Stimulated 1.25 hours upper at 32, lower at 33 per cent
Stimulated 5 hours both sides at 32 per cent

These results indicate that the osmotic pressure of the cell rises as the time of stimulation increases, reaches a maximum at or before visible response, and decreases as the response nears completion. The osmotic pressure is greater on the convex side during the period of response. It is interesting to note that the maximum acidity, as shown in a previous paper (18), is reached in 30 minutes, while the maximum turgor is reached in 45 minutes. The results of different investigators on the turgor change of stimulated shoots

show little agreement. Some writers have found the greater turgor on the concave, some on the convex, side; others have found no difference in turgor in either flank of the stimulated shoot or in the stimulated versus the unstimulated organ, with the balance of the argument rather in favor of the last mentioned. Inspection of the work of those writers who have tabulated their results, however, shows that the time of stimulation (ranging in general from several hours to several days) was too long to catch the change in turgor, which change appears to take place in a relatively short time, as was originally determined by DEVRIES in his macroscopic turgor experiments on geotropically stimulated grass nodes.

Respiration

Qualitative experiments were conducted upon the relative respiration of stimulated and unstimulated roots, and upon the upper and under flanks of geotropically stimulated shoots. These experiments were made in the TASHIRO (19) biometer apparatus, which determines the relative rate of respiration by the precipitation of barium carbonate on the surface of a drop of barium hydrate in a closed chamber.

The roots, without previous stimulation, were placed, one horizontally and one vertically, in similar chambers designated as left and right respectively. The shoots were stimulated for periods varying from 10 minutes to 5 hours. They were split longitudinally just before being placed in the apparatus. The roots were suspended and the shoots were placed horizontally, the upper with the cut surface down, and the lower with the cut surface up, as during stimulation. Both were placed across Van Tieghem cells in order to give equal opportunity for carbon dioxide diffusion. Many seedlings were tested with uniform results.

Table II shows that a geotropically stimulated root has a higher rate of respiration than the unstimulated root, and that in the stimulated shoot the under (convex) side shows a higher rate of respiration than the upper (concave) side at all intervals of time during stimulation and response that were investigated. It also shows that the rate of respiration decreases as the time of stimulation increases.

The effect of geotropic stimulation upon the cell structure of the responded shoot was determined through microscopical examination. Longitudinal sections from concave to convex side of the completely responded shoot in the region of the angle of greatest curvature were cut on the freezing microtome, and camera lucida drawings were made of corresponding areas on the concave and convex flanks of the organ (figs. 4, 5).

TABLE II

RELATIVE RESPIRATION

Seedling	Left chamber	Right chamber	Time stimulated	Time in apparatus	Greater precipitation of $BaCO_3$
Roots					
Sunflower...	Horizontally placed	Vertically placed	.....	4 minutes	Horizontal root
Sunflower...	Vertically placed	Horizontally placed	.....	4 minutes	Horizontal root
Zea Mays...	Horizontally placed	Vertically placed	.....	4 minutes	Horizontal root
Shoots					
Vicia Faba..	Convex side	Concave side	10 minutes	2 minutes	Convex (much greater)
Vicia Faba..	Convex side	Concave side	2 hours, 31 minutes	3 minutes	Convex side
Vicia Faba..	Concave side	Convex side	4 hours, 58 minutes	7 minutes	Convex side

A study of fig. 5 shows that the cells on the convex side are larger than those on the concave side. A 10 cm. square on the convex side contains 40 cells, while a corresponding area on the concave side shows 72 cells. This result is in accord with the work of previous investigators.

Summary

1. The reducing sugars remain constant throughout stimulation and response.

2. The hydrolyzable sugars increase on the convex side at the expense of the polysaccharides as response takes place.

3. The total sugars are constant until beginning of response, when the sugars of the convex side become greater.

4. The osmotic pressure increases until visible curvature has taken place. At the end of the reaction both flanks show the same osmotic pressure, which, however, is greater than that of the normal shoot.

5. Respiration of the geotropically stimulated root is greater than that of the unstimulated organ.

6. The rate of respiration of the convex side of the geotropically stimulated shoot is greater than that of the concave side throughout the period of perception and response.

7. Respiration decreases as the time of stimulation increases.

8. The steps, in point of time, of the chemical changes that take place in a geotropically stimulated shoot are: (1) increased respiration, (2) increased acidity (18), (3) increased turgor, and (4) increased production of hydrolyzable sugars with corresponding decrease of polysaccharides on the convex side of the responding organ.

The writer is greatly indebted to Dr. WILLIAM CROCKER, who suggested the problem, and who gave much assistance during the progress of the work; to Dr. F. C. KOCH for help in the methods of analysis; and to Dr. SHIRO TASHIRO for assistance in the work on respiration.

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BRIEFER ARTICLES

SECTIONING HARD WOODY TISSUES

In preparing hard or refractory woods for sectioning it has been customary to soften in hydrofluoric acid and imbed in either celloidin or gelatin, the latter process being favored in the sectioning of partly disorganized material. In connection with certain anatomical problems, a method of imbedding in paraffin after the demineralization of the woody tissues by means of hydrofluoric acid has been developed, and has proved most successful in dealing with either hard woody tissues or non-homogeneous objects, possessing both soft delicate tissues and hard lignified structures. More uniform results are assured with this method, and it is also possible thus to secure an unbroken series of sections without the tedious and complicated process involved in the celloidin method.

PREPARATION OF MATERIAL.—The woody material is first cut into blocks of a convenient size for sectioning. In the case of hard stems and roots a fine sharp hacksaw should be used to prevent the tearing or jamming of the tissues in the vicinity of the cut surfaces. More delicate material, such as small roots, seedlings, herbaceous stems, etc., may be cut into smaller portions by means of a sharp knife, preferably a Gillette razor blade. If the material to be examined is dead and dry, it should be repeatedly boiled in water and cooled to remove all air from the tissues, as in the celloidin method. If living, it should be well fixed in some suitable reagent. A mixture of formalin, alcohol, and glacial acetic acid has proven satisfactory for most anatomical work: 50 per cent alcohol, 100 cc.; formalin (commercial), 6 cc.; glacial acetic acid, 3 cc. Fix 24–48 hours and rinse thoroughly in running water.

DEMINERALIZATION.—The blocks of material thus prepared are ready for the next step in the process, which is the demineralization and general softening of the lignified structures of the stem by means of hydrofluoric acid. The blocks are transferred directly from water to either 50 per cent aqueous solution of commercial hydrofluoric acid or hydrofluoric acid full strength. The strength of the acid and the length of time in the reagent depend, of course, upon the nature of the material. Cubes of very hard woods of a comparatively homogeneous structure, such as the oak, require 3–4 weeks in 50 per cent hydrofluoric

acid. Pure acid may be used if it is desired to hurry the process, but great care should be taken and the material should be examined often and removed as soon as it cuts easily with a Gillette razor blade. Rhizomes of *Osmunda* and similar material, possessing very hard sheathing leaf bases, may remain in 50 per cent acid several months without apparent injury to other than the most fragile parenchyma tissues. Blocks of *Dioon spinulosum* 1–2 cm. square were treated with 50 per cent acid 3–6 weeks with gratifying results. *Welwitschia*, the delicate parenchyma tissue of which is crowded with rigid spicular cells of great size, after treating with 50 per cent hydrofluoric acid and imbedding in paraffin, can be sectioned without difficulty. *Rhipogonum scandens*, a New Zealand liana, impossible to section by ordinary methods due to the extensive amount of sclerenchymatous tissue distributed through the stem, especially surrounding the scattered bundles, sectioned with perfect ease after immersion for one week in full strength hydrofluoric acid. Non-homogeneous material, such as corn stem, usually difficult to section, especially after it attains a diameter of 1.5–2.5 cm., because of the rigidity of the bundles and the delicate character of the parenchyma, was treated with a 25 per cent solution of hydrofluoric acid for one week, and sections 15–20 μ in thickness were easily cut from 52° C. paraffin. In order to minimize the time of heating of such material in the paraffin bath during the infiltration process, blocks should not average more than 1–1.5 cm. in thickness. The leaves and stem of wheat, oats, and other cereals also contain more or less silica, which makes them very refractory objects to cut, and accounts for the difficulty in obtaining sections of uredospores and teleutospores of *Puccinia graminis*. Immersion of these leaves in 10 per cent solution of hydrofluoric acid for a few days, possibly a week, should remove much of the silica without appreciable injury either to the cell walls or cell contents. After removal from the softening medium, the material should be thoroughly washed in running water to remove all traces of the reagent and then placed in 60 per cent alcohol.

DEHYDRATION AND CLEARING.—In passing through the alcohol and xylol series in the process of dehydration and clearing, the time required for each stage ranges from 12 hours for each of the 60, 70, 80, and 95 grades of alcohol, to 24 hours for the absolute alcohol and each of the absolute alcohol and xylol series. Four grades, 25, 50, 75, and 100 per cent xylol, are generally sufficient. Any air or gases remaining in the tissues should be removed by means of a vacuum pump while the woody material is in pure xylol before the addition of the paraffin.

INFILTRATION WITH PARAFFIN.—During the first 36 hours in the process of infiltration with paraffin the wood is kept *on* the paraffin bath, but shortly before the mixture of xylol and paraffin is replaced with pure melted paraffin; both the material and the paraffin mixture are transferred to a flat dish of some kind to facilitate a quick evaporation of the xylol and then placed *in* the bath. At least two or three changes of paraffin are usually desirable. Special care has to be taken at this point, the best results being obtained when such woody or partially woody material is carried through the final process of infiltration with paraffin (melting point 52° C.) from 48 to 72 hours.

SECTIONING.—With a proper allowance of time for infiltration, sections of the most refractory tissues ranging from 10 to 30 μ in thickness may be cut with a sliding microtome with perfect ease, and a complete series obtained by removing each section, as cut, from the knife and placing it directly upon a slide well coated with albumen fixative and flooded with water. All paraffin sections thus cut and not held in ribbon are likely to curl. To prevent this curling of the section as it comes upon the knife it has been the writer's practice, after flooding the surface of the object and the knife with water (using ice water in warm weather and slightly warmed water in cold weather), to hold a camel's hair brush or preferably the tip of the first finger lightly against the section as it is being cut. The section, unless of considerable size, will then adhere to the moist finger tip and can thus be transferred to the slide without danger of tearing or crushing. With practice sections may be cut and transferred from the microtome knife to the slide very rapidly by this method, and the problem of curling entirely obviated.

Subsequent stages in the fixing of sections to the slide, removal of paraffin, staining and mounting, follow the usual paraffin schedule.—LADEMA M. LANGDON, *University of Chicago*.

CAMPHORINA VS. CINNAMOMUM

In a short article on the botanical nomenclature of the Pharmacopoeia, FARWELL[1] proposes to adopt the generic name *Camphorina* Noronha (1790) in place of *Cinnamomum* Blume (1825), although the latter, originally proposed by TOURNEFORT, had been used by LINNAEUS in the first edition of his *Systema* in 1735. It is not my object to discuss the validity of this proposed change, but aside from calling attention

[1] The Druggists Circular **62**:535. 1918. The first paper of the series was published in Botanical Nomenclature of the U.S.P. IX, *op. cit.* **61**:173–176. 1917.

to the fact that a considerable number of new names have been published in a strictly trade journal where they will probably not be noticed by systematists, attention should be called to the naïve and wholly unnecessary publication of the binomial *Camphorina saigonica* Farwell, a *nomen nudum*, as follows: "The Saigon Cinnamon. *Camphorina Saigonica*, n.sp. The plant producing the Saigon Cinnamon has not as yet been definitely determined, but it is generally supposed to be an undescribed species. The bark is well described in the U.S.P. on pages 114 and 115, and I tentatively propose the above name for the species producing it." In the opening statement it appears as *Cinnamomum saigonicum*, which may also be credited to FARWELL as a *nomen nudum*, although this is the name of the drug used in the 1905–1907 and 1916 editions of the U.S. Pharmacopoeia, where the comment is added: "The bark of an undetermined species of *Cinnamomum*." KRAEMER[2] states that Saigon cinnamon is obtained from *Cinnamomum Loureiri* (?) and other species cultivated in Cochin China and parts of China and exported from Saigon, so that it would appear that the species yielding the product is by no means generally assumed to be an undescribed species as FARWELL indicates.

Knowing from experience the great difficulty of identifying species of *Cinnamomum*, even when complete material is available, I communicated FARWELL'S proposition to Dr. A. CHEVALIER, Director of the Institut Scientifique in Saigon, the following quotation being from his letter of July 21, 1919: "Je vous remercie de m'avoir communiqué un renseignement bibliographique sur la cannelle de Saigon. Il n'existe pas dans le commerce de cannelle de Saigon. Celle qui est exportée par le port de Saigon a été achetée par les marchands chinois en Annam ou elle est fournie par le *Cinnamomum Loureiri* Nees." See also A. CHEVALIER in Bull. Écon., Indochine **22**:526. 1919.

Although *Cinnamomum Loureiri* is not admitted by LECOMTE[3] as an Indo-Chinese species, CHEVALIER is doubtless correct in his identification. From the very fact that 7 species of *Cinnamomum* are definitely known from southern China and that 11 are known from Indo-China, coupled with the fact that the accessible parts of both regions are fairly well explored from a botanical standpoint, it is unreasonable to assume that a commercially important species such as the one under consideration has escaped detection up to the present time.

[2] Botany and pharmacognosy, p. 513. 1910; Scientific and applied pharmacognosy, p. 304. 1915.

[3] Fl. Gén. Indo-Chine **5**:109–117. 1915.

Exception may well be taken to the proposed new names under KAVA,[4] *Piper esculentum* Farwell, *Methysticum methysticum* Farwell, and *Methysticum esculentum* Farwell. These are proposed because *Piper methysticum* Forst. f. (1786) is assumed to be different from *P. methysticum* Linn. f. (1781). Before adopting the new names proposed by FARWELL it would be well to compare the actual types in London, as such comparison will probably show *Piper methysticum* Linn. f. and *P. methysticum* Forst. f. to be identical and based on material of the same (Forster's) collection. At any rate it would seem to be wholly unnecessary to publish *Piper esculentum* Farwell and *Methysticum esculentum* Farwell for the same species in the same article merely because there is a considerable difference of opinion among botanists as to the generic status of the plant in question. A taxonomist should be able to determine to his own satisfaction the status of a proposed genus before making transfers to it.—E. D. MERRILL, *Bureau of Science, Manila, P.I.*

[4] FARWELL, O. A., Botanical nomenclature of the N[ational] F[ormulary] IV, *op. cit.* **61**:229–232. 1917. There is a continuation of this paper, *op. cit.* **63**:49, 50. 1919.

CURRENT LITERATURE

NOTES FOR STUDENTS

Taxonomic notes.—ENGLER[1] has described a new genus (*Hieronymusia*) of Saxifragaceae from South America (Argentina and Bolivia). It was referred first to *Saxifraga* (*S. alchemilloides*) and later to *Suksdorfia*.

SCHLECHTER,[2] in continuation of his studies of orchids, has described 19 new species, representing 9 genera, chiefly collected in Argentina by DUSEN.

ST. JOHN[3] has described a new genus (*Phanerotaenia*) of Umbelliferae, based on *Polytaenia Nuttallii texana* C. and R., of Texas and Oklahoma.

SETCHELL and GARDNER[4] have begun the publication of the marine algae of the Pacific Coast, the first part including the blue-green algae. The group as presented contains 30 genera, representing 6 families. Of the 93 species included, 35 are described as new.—J. M. C.

Polycotylous seedlings.—Miss BEXON[5] has found that *Centranthus ruber* (Valerianceae) develops a remarkably high proportion of polycotylous seedlings. From two square yards of soil in which self-sown seeds of this species were germinating, 87 polycotylous specimens were obtained and studied. They are grouped as hemitricotyls, tricotyls, hemitetracotyls, and tetracotyls, terms which are self-explanatory. The vascular anatomy of these various conditions was investigated, with some interesting results. A "twinned" seedling was of special interest, the twinning being due either to the fusion of two distinct embryos, or to the partial separation of the product of the embryo initial.—J. M. C.

Unusual monocotyledonous roots.—Miss SPRATT[6] has discovered some interesting anomalies in the roots of some monocotyledons. In *Dracaena* there

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[1] ENGLER, A., *Hieronymusia* Engl., eine neue Gattung der Saxifragaceen. Notizbl. Königl. Bot. Gart. **7**:265–267. *fig. 1*. 1918.

[2] SCHLECHTER, R., III. Orchidaceae novae, in caldariis Horti Dahlemensis cultae. Notizbl. Königl. Bot. Gart. **7**:268–280. 1918.

[3] ST. JOHN, H., *Phanerotaenia*, a new genus of Umbelliferae. Rhodora **21**:181–183. 1919.

[4] SETCHELL, W. A., and GARDNER, N. L., The marine algae of the Pacific Coast of North America. I. Myxophyceae. Univ. Calif. Publ. Bot. **8**:1–138. *pls. 1–8*. 1919.

[5] BEXON, DOROTHY, Observations on the anatomy of some polycotylous seedlings of *Centranthus ruber*. Ann. Botany **34**:81–94. *figs. 9*. 1920.

[6] SPRATT, AMY VERA, Some anomalies in monocotyledonous roots. Ann. Botany **34**:99–105. *pl. 3. fig. 1*. 1920.

are two kinds of secondary vascular development; in the one case the pericycle becomes meristematic and adds vascular elements to the stele; and in the case of large roots this is followed by a similar behavior of the cortical cells just outside the endodermis. In *Pandanus* and *Yucca* internal vascular strands were discovered, which are differentiated at the growing point. In the aerial roots of certain Araceae, inside of the radial primary vascular cylinder, there occur scattered groups of xylem and phloem vessels.—J. M. C.

Sex intergrades.—YAMPOLSKY[7] has brought together the evidence of sex intergrades in flowering plants. The discussion is based upon his results with *Mercurialis annua*, in which he had found sexuality to be a fluctuating character. At the close of the paper he gives a long list of families showing transition from the monoecious to the dioecious condition, and also tabulates the results under 12 types. The general conclusion confirms the view that the potentialities of both sexes exist in all plants, and are not localized in any particular region or cells.—J. M. C.

Endosperm development in Vaccinium.—STEVENS[8] has described an interesting case of endosperm development in *Vaccinium corymbosum*. Two distinct types of initial endosperm development recognized are free nuclear division and wall formation, and these types have been regarded as characteristic of different genera and even families. In *V. corymbosum*, from material collected from a single plant, STEVENS finds that endosperm development may begin either way. This emphasizes the fact that many of our morphological categories have been too rigidly defined.—J. M. C.

North American flora.—The second part of volume 24 chiefly consists of the completion of the genus *Parosela*, by RYDBERG, 43 species of which had been published in the preceding part.[9] This genus is recognized to include 178 North American species, 46 being described in the present contribution as new, and many being species transferred from other genera. The three other genera presented are *Thornbera* (13 species, 5 new), *Petalostemon* (42 species, 8 new), and *Kuhnistera* (2 species).—J. M. C.

New species of Piper.—In connection with the biological survey of Panama, conducted several years ago by the Smithsonian Institution, the collected material of the Piperaceae was sent to the late CASIMIR DECANDOLLE, whose determinations have just been published.[10] The collection was found to include 27 new species of *Piper* and 4 new varieties.—J. M. C.

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[7] YAMPOLSKY, CECIL, The occurrence and inheritance of sex intergradation in plants. Amer. Jour. Bot. **7**:21–38. 1920.

[8] STEVENS, N. E., The development of the endosperm in *Vaccinium corymbosum*. Torr. Bot. Club **46**:465–468. *figs.* 4. 1919.

[9] BOT. GAZ. **68**:65. 1919.

[10] DECANDOLLE, CASIMIR, New species of *Piper* from Panama. Smithsonian Miscell. Coll. **71**: no. 6. pp.17. 1920.

VOLUME LXX NUMBER 2

THE

BOTANICAL GAZETTE

AUGUST 1920

STUDIES IN THE GENUS BIDENS. V

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 269

EARL E. SHERFF

(WITH PLATES XI–XIV)

Bidens exigua, sp. nov. (pl. XI).—Herba annua, 1.5–3 dm. alta; caule tenuissimo, subrecto, striato, subsimplici. Folia opposita (summa alternata), membranacea, petiolata, petiolo adjecto 3–5 cm. longa, bipinnata, glabra; foliolis (3 aut 5) maximam partem 3–5-partitis; lobis integris, subobtusis, infirme apiculatis. Petioli 0.5–2 cm. longi, basi connati. Capitula pauca aut solitaria, discoidea, tenuiter pedunculata pedunculis 2–5 cm. longis, ad anthesin 5–6 mm. longa et 1.5–2 mm. lata infra, 2–3 mm. lata supra; in fructu, circ. 9 mm. longa et 2–5 mm. lata. Involucrum basi sparsim hispidum aut glabratum; bracteis duplici serie dispositis; exterioribus (4–7) linearibus, 2–3 mm. longis, ciliatis, ad faciem glabris aut pubescentibus, ad apicem induratis; interioribus dimidio longioribus, glabratis, lanceolatis, striatis, margine diaphanis. Achaenia (submatura) linearia, glabra aut supra ad margines remote hispida, bi- aut triaristata aristis retrorsum hamosis hamis tenuibus, 4–8 mm. longa.

SPECIMENS EXAMINED.—*C. H. T. Townsend* 1513, alt. 1607 m., Chosica Canyon, Peru, April 20, 1913 (Herb. U.S. Nat. Mus., no. 602943, type).

The plants of the type sheet would seem at first to be merely depauperate or impoverished forms of some species normally larger and perhaps already described. The technical characters, however, do not match those of any other species known to me. There are several species very close to *B. exigua* all of

which might once easily have passed for *B. bipinnata* L., yet which, with subsequent advances in our knowledge, have been proved indisputably to be distinct and severally valid. Among these are *B. heterosperma* Gray and *B. Lemmoni* Gray of the southwestern United States and Mexico, and *B. parviflora* Willd. of Asia. *B. exigua* is nearest to *B. Lemmoni* and *B. parviflora;* like these, it may well be expected to prove constant and worthy of specific rank.

Bidens duranginensis, sp. nov. (pl. XI).—Herba annua, glabrata, demum circa 6–9 dm. alta; caule subtetragono, ramis acute tetragonis, ramis acute tetragonis, longis et tenuibus, striatis, infra minute pubescentibus. Folia opposita, petiolata, petiolo adjecto 10–12 cm. longa (eis ramorum 1–3 cm. longis), pinnata, serrata aut dentata (aut etiam inciso-dentata), ciliata, foliolis ovatis aut ovato-lanceolatis, saepe duobus aut quatuor imis cuiusque folii tripartitis. Petioli (foliorum caulis) 2.5–4 cm. longi, ad basim ciliati et connati. Capitula multa, subligulata, tenuiter pedunculata, pedunculis 3–8 cm. longis, ad anthesin 4–7 mm. alta et (liguiis adjectis) 0.8–1.3 cm. lata, in fructu 1.2–1.4 cm. alta et 6–8 mm. lata. Involucrum basi hispidum, bracteis duplici serie dispositis; exterioribus (circ. 8) linearibus, fere glabris, apice induratis, 2–3 mm. longis; interioribus dimidio longioribus, anguste lanceolatis; membranaceis, margine diaphanis. Ligulae (3–6) subalbidae, anguste ovatae, 4–7-striatae, 4–6 mm. longae. Achaenia linearia, nigra, glabra aut supra sparsim hispida, 2–4-aristata aristis flavis et retrorsum hamosis, corpore 6–12 mm longa.

SPECIMENS EXAMINED.—*Dr. Edward Palmer* 756, west side of Iron Mountain, vicinity of city of Durango, Durango, Mexico, October 1896 (Herb. Gray, type; Herb. Field Mus., no. 51825; Herb. U.S. Nat. Mus.); *idem* 612, vicinity of city of Durango, Durango, Mexico, April to November 1896 (Herb. Field Mus., nos. 51704 and 51705; Herb. Gray, differing from type material, apparently, merely in being somewhat younger).

This species, if we attempt to delimit it in a taxonomic way, is confessedly of unsatisfactory status. The type collection had been determined as *Bidens anthriscoides* DC., but the plants are very different from the type material of that species (*Berlandier* 1010, Herb. Brit. Mus.; Herb. Drake, Paris). It is manifestly an ally of *B. pilosa* L., from which it differs in its whitish rays and in its foliage, the lower leaflets of the stem leaves tending to be distinctly tripartite. This last character distinguishes it likewise from *B. leucantha* (L.) Willd. In foliage characters it slightly simulates *B. chinensis* (L.) Willd. of

the Orient and *B. subalternans* DC. of South America. I have seen two specimens by E. O. WOOTON from New Mexico (Mesilla Valley, Dona Ana County, October 1895, U.S. Nat. Herb., nos. 561445 and 663170; referred to *B. anthriscoides* DC. by WOOTON and STANDLEY, Contrib. U.S. Nat. Herb. 19:704. 1915) that are evidently true *B. bipinnata* L., yet which approach *B. subalternans* DC. A third plant, also by WOOTON (Las Cruces, New Mexico, October 1895, Herb. N.Y. Bot. Gard.), approaches *B. subalternans* DC. in foliage still more, but is nevertheless clearly a form of *B. bipinnata* L. All three of these plants are suggested by the type of *B. duranginensis*. They appear, however, to be entirely distinct in a specific way. Future field studies, to determine the range of variation and the limits of demarcation for the Durango plants, are highly desirable.

No other group in the genus *Bidens* has been so badly neglected heretofore, considering the number of species involved, as has that group native to the Hawaiian Islands and other islands of the Pacific, and, by some authors, segregated as a separate genus, *Campylotheca*. Nearly a century ago GAUDICHAUD (Voy. Freycinet Bot. 464. *pl. 85*. 1826–1829), describing a species collected in the Hawaiian Islands during FREYCINET'S voyage, named the plant *Bidens micrantha*. Shortly afterward CASSINI (Dict. Sci. Nat. 51:475. 1827) called attention to the curved achenes of GAUDICHAUD'S species. He made this achenial character the basis for proposing his new genus *Campylotheca*. Later LESSING (Linnaea 6:508. 1831) accepted CASSINI'S genus for species like *Bidens micrantha* Gaud., but he erected a new genus, *Adenolepis*, to include a somewhat different form. I propose to discuss *Adenolepis* in a future article. Concerning *Campylotheca*, however, we may proceed to note that the name was retained by DE CANDOLLE in his *Prodromus* (5:593. 1836), although elsewhere it was accorded only slight attention. In fact, the collections in those days embraced so few specimens from the Pacific Islands that little study was made of the Pacific flora by taxonomists. NUTTALL, in 1841 (Trans. Amer. Phil. Soc. N.S. 7:368), reduced *Campylotheca* to the rank of a section under *Bidens*, but did not give extended reasons for so doing. His attention had been directed to the subject by his having traveled among the Hawaiian Islands and discovered there at least one new species of *Bidens* (*B. gracilis*). NUTTALL, however, did evince a rejection of CASSINI'S main character for *Campylotheca*,

namely, the curved or twisted achenes. He worded his description to read "sometimes curved or contorted," and for one species (*B. mutica*) he definitely described the achenes as "straight." Since NUTTALL'S time, we may add, many other closely affiliated species have been discovered, including forms of *Bidens micrantha* itself, which have straight achenes, thus bringing the curved-achene character into discredit. In 1856 SCHULTZ BIPONTINUS undertook the determination of various specimens collected on Nukahiva by EDWARD JARDIN. Finding four new species native to this single small island, SCHULTZ BIPONTINUS appears to have entered upon a very careful and painstaking research into the subject of their generic affinities, finally publishing his results[1] (Flora **39**:357. 1856). As regards the maintenance of a genus *Campylotheca* apart from *Bidens*, he was unreservedly against such a course. His four new species from Nukahiva and all of the Hawaiian species he referred to *Bidens*.

In my own attempts accurately to evaluate SCHULTZ BIPONTINUS' opinion, I sought four years ago to repeat his studies upon the Nukahiva species. Through the generous assistance of M. ST. AHNNE, President of the Chamber of Agriculture of Tahiti, and the careful, persistent search by his friend, M. HENRY, President of the French Alliance of Nukahiva,[2] I have been able to secure many mature achenes and herbarium specimens from the same island where JARDIN originally collected. Achenes of each kind were planted, and thus, during a period of three years, several hundred live plants have been obtained for observation. Having supplemented in this way my examination of the few herbarium specimens available, I have been able to match all of SCHULTZ BIPONTINUS' four descriptions very well. The four species (*Bidens cordifolia*, *B. polycephala*, *B. serrulata*, *B. Jardinii*) are clearly distinct in leaf characters of the older plants and in fruit characters. More-

[1] For a personal estimate, apparently unbiased and accurate, of the taxonomic ability and sagacity that SCHULTZ BIPONTINUS displayed at times, see BENTHAM, Jour. Linn. Soc. **13**:340. 1873.

[2] I cannot too gratefully thank M. ST. AHNNE and M. HENRY for their great kindness shown to me during the progress of my work. Repeatedly they have assisted in procuring for me the very materials that were essential for a correct understanding of the far away Pacific Island flora.

over, none of the four is found to differ generically from the various Hawaiian species, both groups even emitting the same peculiar carrot-like odor when the leaves are bruised. There can remain no doubt, therefore, regarding the exact basis of SCHULTZ BIPONTINUS' study. Furthermore, the scholarly and critical way in which he attacked the entire subject must needs inspire a strong sense of confidence in his judgment and in the course pursued by him in equating *Campylotheca* with *Bidens*.

In 1861 ASA GRAY (Proc. Amer. Acad. **5**:125–128) made the next important contribution to a knowledge of the group. GRAY had received from the Museum of Natural History in Paris several specimens collected by M. J. REMY in the Hawaiian Islands, also a number from the United States Exploring Expedition under Captain WILKES, collected in the Hawaiian Islands, Tahiti, Eimeo, and elsewhere in the Pacific. Most of these were new species. GRAY'S publication indicates that he was probably unaware of SCHULTZ BIPONTINUS' paper. Thus, for example, he inadvertently created the name *Coreopsis Macraei* for a plant already named by the latter *Bidens Campylotheca*. As, therefore, he does not seem to have read SCHULTZ BIPONTINUS' paper, it is all the more interesting and valuable to find that GRAY, too, was compelled to abandon the name *Campylotheca*. Species having the achenes wingless and the awns retrorsely barbed he described under *Bidens*. But several other species, different in having either exaristate achenes or even winged achenes, he described under *Coreopsis*. Thus he described *Bidens hawaiensis*, *B. lantanoides*, *Coreopsis mauiensis*, *C. macrocarpa*, *C. Macraei*, *C. cosmoides*, and *C. Menziesii*. GRAY'S own words at the time of describing some of these species are worthy of note. Speaking of the futility of maintaining *Campylotheca* as a separate genus, apart from *Bidens* and *Coreopsis*, he said: "Its adoption merely gives us three limitless genera unmarked by any peculiarity in habit, in the place of two artificially separated ones. . . . . Vain is the attempt to draw absolute limits where Nature luxuriates in gradations" (Proc. Amer. Acad. **5**:126. 1862).

In 1888 there appeared the posthumous *Flora of the Hawaiian Islands* by WILLIAM HILLEBRAND. HILLEBRAND, from his twenty years of resident study in the Hawaiian Islands and his careful

investigations subsequently, was eminently well versed in their species. His treatment assumes almost the aspect of a monographic revision, and it is evident that he possessed much more than an ordinary knowledge of *Bidens* and related groups. His brilliancy, however, appears to have been manifested, as is so apt to occur with a local botanist, less in the excellence of his genus concept than in that of his species concept. And, even in the latter respect, his generalizations are often necessarily faulty because of the inadequacy of his material. HILLEBRAND, like GRAY, appears never to have seen SCHULTZ BIPONTINUS' paper. He discarded GRAY'S treatment, however, and adopted once again CASSINI'S name *Campylotheca*. Speaking of *Campylotheca* he says (p. 211): "The genus, as it presents itself now, stands evidently nearer to *Bidens* than to *Coreopsis*, and might be merged in the former if it were not for the winged achenes of so many species,[3] which, if admitted in the character of *Bidens*, would efface the limits between that genus and *Coreopsis*." GRAY'S Hawaiian *Bidens* is transferred by HILLEBRAND to *Campylotheca*.

This effort to break down the genus *Bidens* into smaller units is not the first of its kind. As early as 1790,[4] NECKER (Elem. Bot. 1:86–87) subdivided the genus into two new genera. For these he proposed the names *Pluridens* and *Edwarsia;* the first group to include those species with simple foliage (for example, *Bidens cernua* L.), the second to include those species with foliage dissected (for example, *B. pilosa* L. and *B. pinnata* L.). In 1794, MOENCH (Meth. 569 and 595) followed NECKER'S treatment essentially, but substituted the names *Bidens* and *Kerneria* for NECKER'S two names. Neither NECKER'S treatment nor that of MOENCH is today accepted by botanists. In 1836, DE CANDOLLE (Prodr. 5:633) described a new plant that resembled *Bidens*, but which appeared remarkable in having the ligules pistillate and fertile. DE CANDOLLE created the genus *Delucia* therefor, and his new plant he named *Delucia ostruthioides*. Later SCHULTZ BIPONTINUS (Seem. Bot. Voy. Herald 308. 1852–1857) renamed the species *Bidens ostruthioides*, and this latter name has been widely accepted

[3] Regarding the inaccuracy of this statement, cf. footnote 8.

[4] Cf. E. L. GREENE, Pittonia 4:245. 1901.

ever since.[5] In 1901, GREENE (Pittonia **4**:242–270) presented the results of a study of *Bidens*. He commented upon the dissimilarity between such species as *B. cernua* L. and *B. tripartita* L. Even so radical a botanist as he, however, refrained from proposing a generic segregation of the *B. cernua* forms. Nevertheless, GREENE did segregate the aquatic *Bidens Beckii* as the type of a new genus, *Megalodonta;* and, when the peculiar achenes of this species are considered, it seems wise to accept GREENE'S new genus as valid.

Strangely enough, no one appears to have tried to segregate generically the pronounced and well defined group of *Bidens* species typified by the species *Bidens reptans* (L.) G. Don.[6] These species differ from the more typical species in being climbers, and in having long flat achenes that are hispid along the two edges in such a way at times as to suggest a centipede. Again, my own *Bidens mirabilis* (BOT. GAZ. **61**:496. *pl. 31*. 1916), with achenes flat, strongly constricted above into a thick neck and crowned with even 8–10 aristae, might be segregated as the type of a new genus. Similarly, the anomalous *Bidens clarendonensis* Britton, with trailing, somewhat woody stem, and thick, rhombic-ovate leaves, would be interpreted by some as representing a new genus.

Thus it is seen that, if we accept the narrow concept of *Bidens* held by CASSINI, LESSING, and HILLEBRAND, and seek to segregate the native Pacific species under the name *Campylotheca*, to be consistent we shall have to subject the entire genus *Bidens* to a process of subdivision and segregation, resulting in some six or eight genera. There are at least two good reasons for not adopting such a course. In the first place, the accuracy of such a series of interpretations is not so well established as to justify overturning almost the entire nomenclature of the genus. In the second place, the lines of demarcation among the various subordinate groups are

[5] In the herbaria *Bidens ostruthioides* is the universally used name. It is interesting to note that a closely similar form was described by KLATTE as *Bidens guatemalensis* (Bot. Jahrb. **8**:44. 1887). Another related form, apparently more clearly distinct, however, was placed by BENTHAM in *Bidens* and described as *B. costaricensis* (Benth. ex Oerst., Kjoeb. Vidensk. Meddel. 94. 1852).

[6] DE CANDOLLE (Prodr. **5**:599. 1836), however, did create the name *Bidens Coreopsidis* for one of these species. And, even earlier, the names *Coreopsis reptans* L., *Coreopsis incisa* Ker., etc., had been given to certain of these species, but without very serious consideration being given to their generic affiliations.

so fluctuating and inconstant that efforts to apply a binomial system of nomenclature to the many species would be rendered even much more difficult than before. I am constrained to reject, therefore, any idea of seriously interfering with the general status of *Bidens*. CASSINI'S name *Campylotheca* I am compelled to discard.[7]

Having laid aside the name *Campylotheca*, there remains one further matter with which to deal. As stated previously (BOT. GAZ. **59**:308. 1915), we find among the numerous species of *Bidens* and the allied genus *Coreopsis* "no absolute uniformity in even one distinctive character. However, one such character does persist to a surprising extent. It is the presence (in *Coreopsis*) or absence (in *Bidens*) of two lateral wings upon the mature achene. Among so many species from widely remote regions does this character separate two genera with different aspects that, *in cases where other criteria are absent*, it appears to offer the only logical basis of distinction." This presence or absence of achene wings was given great weight by GRAY, but in the Pacific flora the wing character is unreliable, and will lead, if absence of wings be demanded from all species of *Bidens*, to an arbitrary and unnatural arrangement. Some three or four Hawaiian forms commonly have accessory awns or barbs below the achene's apex, and either these or the principal awns frequently are decurrent along the achenial edges as a more or less thickened margin or even as a wing; or at times the awns seem unrelated to the wings. In "*Coreopsis mauiensis*" Gray, these wings are very conspicuous. The number of Hawaiian

[7] In taking this step it is reassuring to read the words of so eminent a student of the Compositae as BENTHAM. Speaking of CASSINI and his work, he stated (Jour. Linn. Soc. **13**:338. 1873): "Unfortunately, however, in working out the details of the genera in the 'Dictionnaire,' he indulged in an enormous and useless multiplication of generic names, which only tended to throw the nomenclature into confusion, and cast a slur upon all his labors. Wherever he observed a slight difference in the involucre, pappus, or general aspect, or could not readily identify an imperfect specimen, an engraved figure, or a description often incorrect, he at once set it down as a new genus, and has thus, more than any other botanist of equal ability, overloaded the science with useless synonyms. So recklessly, indeed, did he give way to this mania of coining new names, that he on many occasions proposed two, or even three, for the same genus, leaving future botanists to take their choice." CASSINI did not neglect *Campylotheca* in this respect. At the very outset he proposed *Dolicotheca* as an alternative name. This latter name, however, was never adopted by LESSING, DE CANDOLLE, or others.

species that exhibit this character, however, is very small compared with the remaining Pacific species that lack it.[8] Moreover, a study of their other characters, such as odor of bruised foliage (when fresh) and shape of ligules, as well as range of distribution, shows them to be much closer to the wingless-achened *Bidens* species of the Pacific than to the wing-achened American species, *Coreopsis lanceolata* L., that must be taken as the type of the genus *Coreopsis*. It seems wise, therefore, to transfer such species directly to *Bidens* rather than leave them with *Coreopsis*, where originally placed by GRAY. We shall have even then no greater incongruity in *Bidens* than must perforce be tolerated in *Coreopsis*. Thus, for example, all authors who have dealt with the subject have retained the North American wingless-achened *Coreopsis rosea* Nutt. and *C. tinctoria* Nutt. in *Coreopsis* despite their anomalous achenes, because their other characters clearly indicated a closer affinity with *Coreopsis*. Manifestly this was the only correct course to pursue, and my own procedure is precisely comparable.

In the following list, therefore, such transfers are made. In addition, there are transferred certain other species that were described by GRAY under *Coreopsis* (where he placed them because they lacked retrorsely barbed awns; cf. BOT. GAZ. **59**:305–308. 1915), or by HILLEBRAND under *Campylotheca*. The new names are:

Bidens molokaiensis (Hillebr.), comb. nov.—*Campylotheca molokaiensis* Hillebr., Fl. Hawaiian Isls. 212. 1888.

Bidens macrocarpa (Gray), comb. nov.—*Coreopsis* (*Campylotheca*) *macrocarpa* Gray, Proc. Amer. Acad. **5**: 126. 1862.

Bidens Remyi (Hillebr.), comb. nov.[9]—*Campylotheca Remyi* Hillebr., *loc. cit.*, 212; *Coreopsis Hillebrandiana* Drake del Cast., Illustr. Fl. Ins. Mar. Pacif. 209. 1890.

[8] Cf. HILLEBRAND'S misleading words, "the winged achenes of so many species." Doubtless HILLEBRAND was recalling many specimens of a few species, and unguardedly referring to them as "so many species." Reference to his individual descriptions shows few of the species to be described as wing-achened.

[9] This species was based by HILLEBRAND upon *M. J. Remy* 287, a single specimen in Gray Herbarium. I have seen not only the type but an excellent duplicate in Paris (Herb. Mus. Hist. Nat.), also fine specimens collected by *Faurie* (Herb. Brit. Mus.), *Forbes* (Herb. Bernice Pauahi Bishop Mus.), etc. The species should not be confused with *Bidens Remyi* Drake del Cast. (Illustr. Fl. Ins. Mar. Pacif. *pl. 39*. 1888; *Coreopsis Remyi* Drake del Cast., *loc. cit.*, 210), a species founded upon *M. J. Remy* 281,

Bidens dichotoma (Hillebr.), comb. nov.—*Compylotheca dichotoma* Hillebr., *loc. cit.*, 212.

Bidens mauiensis (Gray), comb. nov.—*Coreopsis mauiensis* Gray, Proc. Amer. Acad. **5**:125. 1862; *Campylotheca mauiensis* Hillebr., *loc. cit.*, 213.

Bidens cosmoides (Gray), comb. nov.—*Coreopsis* (*Campylotheca*) *cosmoides* Gray, *loc. cit.*, 126.

Bidens Menziesii (Gray), comb. nov.—*Coreopsis* (*Campylotheca*) *Menziesii* Gray, *loc. cit.*, 127.

A most remarkable feature of the flora of the Hawaiian Islands is the large number of endemic species. For a number of years botanists have been cognizant of this pronounced degree of endemism (cf. HILLEBRAND, Fl. Hawaiian Isls. pp. xv and xxv. 1888; MACCAUGHEY, Amer. Botanist **22**:45–52. 1916; BOT. GAZ. **64**:89–114. 1917; *loc. cit.*, **66**:273–275. 1918). Furthermore, the scanty supply of Hawaiian specimens available in most herbaria often makes a proper interpretation of the various endemic forms practically impossible at the present day.

In 1917, almost in despair of being able to arrive at satisfactory opinions respecting several Hawaiian species of *Bidens*, I appealed to certain botanists resident there for aid. One of these, Professor CHARLES N. FORBES, Curator of Botany at the Bernice Pauahi Bishop Museum in Honolulu, proved able to render me assistance of the utmost value. In 1919 he placed at my complete disposal the entire *Bidens* collection of the Bishop Herbarium, also a set of duplicates (later deposited in Field Museum, Chicago). Among these were specimens not only by HILLEBRAND, MANN and BRIGHAM, and other older collectors, but also by FORBES, BRYAN, STOKES, and others of the present century. A considerable portion had

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but which clearly is a mere form of *Bidens micrantha* Gaud. A specimen of *Remy* 281 in Gray Herbarium had been erroneously determined by ASA GRAY as being *Bidens sandvicensis* Less. var. *heterophylla* Gray. Later, HILLEBRAND (*loc. cit.*, 216), having seen this sheet at Gray Herbarium and assuming GRAY'S determination to be correct, naturally equated GRAY'S *B. sandvicensis* var. *heterophylla* with *B. micrantha* Gaud. But the true *B. sandvicensis* Less. var. *heterophylla* Gray was based upon a plant in Kew Herbarium collected by BEECHEY on the Island of Oahu, and treated by HOOKER and ARNOTT as *Bidens luxurians*. This plant of BEECHEY'S is wholly distinct from *B. micrantha* Gaud. and from our *B. Remyi*.

been collected in localities never before visited by botanists. Many of the plants collected even from the better known localities were much superior in point of maturity and state of preservation to those previously collected by other botanists. No less than eleven species were found to be new. Still other species, while not new to science, were represented in such excellent or numerous forms that more elaborate descriptions and more accurate concepts were possible than when the species were first described. The descriptions, with lists of specimens examined, are presented herewith.

Bidens cervicata, sp. nov.—Glabra, supra herbacea, infra forsan suffruticosa, caule acute tetragono, ramoso, ±8 dm. alto. Folia membranacea, pinnata aut summis tripartita, petiolis adjectis 7–15 cm. longis, foliolis lanceolatis, acuminatis, serratis dentibus acribus et tenuiter mucronatis, sparsim ciliatis, 2.5–9 cm. longis et 0.8–2.8 cm. latis, petiolis tenuibus 1.5–4 cm. longis. Capitula multa, subcorymbosa, ligulata, ad anthesin 5–7 mm. alta et 1.5–1.8 cm. lata. Involucri bracteae exteriores plerumque 5, lineares, glabratae, patentes aut reflexae, 1.5–2.5 mm. longae, interioribus multo breviores. Ligulae circ. 5, flavidae, ovato-lanceolatae vel elliptico-oblongae, apice saepe profunde et acriter dentatae, 7–9 mm. longae. Achaenia tenuiter linearia, nigra, exalata, exaristata, glabra aut 1-paucis setis munita, torta, infra angustata, supra cervici-elongata, 1–1.3 cm. longa.

SPECIMENS EXAMINED.—*C. N. Forbes* 1085 K, Waimea Drainage Basin, west side, Kauai, July 3 to August 18, 1917 (Herb. Bishop Mus., type; Herb. Field Mus., no 485172).

Bidens amplectens, sp. nov.—Herbacea supra, infra verisimiliter suffruticosa, ramosa, caule ramisque tetragonis, glabra, probabiliter 5–8 dm. alta. Folia plerumque pinnata, membranacea, petiolis adjectis 4–12.5 cm. longis et 3–7.5 cm. latis; foliolis 3–5, ovato-lanceolatis, serratis dentibus orbiculatis, ad apicem acuminatis, terminali saepe maiore, petiolis tenuibus 2–4 cm. longis. Capitula non multa, sub-solitaria in pedunculis, laxissime corymbosa, adolescentia iis Cosmi specierum non dissimilia, florescentia 6–8 mm. alta et 3–3.5 cm. lata. Involucri bracteae exteriores 5–6, valde reflexae, crassiusculae, lineari-oblanceolatae, ad apicem

subacutae et glanduloso-apiculatae, 3–6 mm. longae, quam interiores paulo breviores. Ligulae 7–8, anguste obovatae, apice obscure dentulatae, 1.5–1.8 cm. longae. Achaenia submatura nigra, plana, exalata, marginibus apiceque setulosa, exaristata aut obscurissime biaristata, circ. 8 mm. longa.

A plant of peculiar aspect, embracing habital characters of *Cosmos* and *Coreopsis* as well as of *Bidens*, hence the name *amplectens*.

SPECIMENS EXAMINED.—*C. N. Forbes* 1839 O, Kawaihapai, Waianae Range, Oahu, Hawaiian Islands, *sine tempore legendi* (Herb. Bishop Mus., type; Herb. Field Mus., no. 485361).

Bidens micranthoides, sp. nov.—Herba glabrata, infra suffruticosa, supra ramosa, ramis gracilibus, 3–5 dm. alta. Folia pinnata aut rarius ternata, petiolis adjectis 3–7 (–12.5) cm. longis et 2–5 (–8) cm. lata, foliolis ovato-lanceolatis aut raro ovatis, serratis, ad apicem plerumque acutis aut etiam longissime acuminatis, nunc membranaceis, nunc subrugoso-crassiusculis, foliolis imis raro tripartitis, petiolis tenuibis 1–5 cm. longis. Capitula supra folia exserta, laxe corymbosa, ad anthesin 5–7 mm. alta et 1.5–2.5 cm. lata. Involucri bracteae exteriores 5–7, lineares, ad apicem subobtusae, glabratae aut glanduloso-pulverulentae, 1–2.5 mm. longae, interioribus multo longioribus. Ligulae 4–6, flavae, ovato-oblanceolatae, ad apicem 2–4-dentulatae, circa 1 cm. longae. Achaenia linearia, exalata, supra et ad margines sparsim setosa, apice setoso-coronulata et biaristata aristis retrorsum hamosis aut saepe plus minusve exaristata, 7–9 mm. longa.

SPECIMENS EXAMINED.—*Captain Beechey*, Oahu (May 19–30, 1826, *fide* Hook. and Arn., Bot. Beech. Voy. p. i. 1841) (Herb. Hookeri in Herb. Kew); *C. N. Forbes* 494 K, Wailua Falls, Kauai, October 5, 1916 (Herb. Bishop Mus.; Herb. Field Mus., no. 485156); *idem* 592 K, Nonou Mountains, Kauai, October 16–17, 1916 (Herb. Bishop Mus.; Herb. Field Mus., no. 485160); *idem* 704 K, Haupu Range, above Nawiliwili Bay, Kauai, October 31, 1916 (Herb. Bishop Mus., type); *idem* 1405 O, Manoa Valley, Oahu, November 23, 1909 (Herb. Bishop Mus.; Herb. Field Mus., no. 485254); *idem* 1849 O, Waiolani Ridge, Oahu, October 27, 1913 (Herb. Bishop Mus.); *idem* 2014 O, ridge east of Kuliououiki, Oahu, November 17, 1914 (Herb. Bishop Mus.).

As the name suggests, this species resembles more or less *B. micrantha* Gaud. In some cases the resemblance in foliage is very deceiving. The preceding specimen by *Beechey* had been determined as *B. micrantha* by HOOKER and

ARNOTT (cf. Hook. and Arn., Bot. Beech. Voy. 86. 1841), although in this case the foliage was very distinct from that of GAUDICHAUD's plate for *B. micrantha*. ASA GRAY, who later studied the *Beechey* plant, referred it incorrectly to *B. sandvicensis* Less. (cf. Gray, Proc. Amer. Acad. **5**:128. 1862). From both *B. micrantha* and *B. sandvicensis* my species differs most noticeably in habit, being lower in stature, apparently more open in its branching, and certainly with the inflorescence much more open, the heads being variously scattered and at different levels, not so corymbose.

Bidens Stokesii, sp. nov. (pl. XII).—Supra herbacea, infra verisimiliter fruticosa, glabra, caule subtetragono, ramoso, ±6 dm. alto. Folia ternata aut 5 foliolis pinnata, membranacea, non ciliata, petiolis adjectis 4–9 cm. longa et 2.5–6 cm. lata, foliolis rhomboideo-ovatis aut lanceolatis, terminali interdum breviter acuminato, orbiculato-serratis, raro inciso-lobulatis, petiolis tenuibus 1.5–4 cm. longis. Capitula pauca, paniculato-corymbosa, tenuiter pedunculata (ad fines ramorum 10–14 cm. nudorum) pedunculis 1–5.5 cm. longis, ligulata, ad anthesin circ. 7 mm. alta et 2–2.5 cm. lata. Involucri bracteae exteriores circ. 8, lineares, glabratae aut sparsissime hispidae, apice indurato, 3–4 mm. longae, erectae aut recurvatae, interioribus longiores. Ligulae 6–7, flavidae, oblongae, apice obscure dentulatae, 7–10 mm. longae. Achaenia linearia, nigra, glabra, interdum plano-marginata sed non vere alata, saepe biaristata aristis tenuibus et obscure retrorso-hamosis, ±7 mm. longa.

SPECIMENS EXAMINED.—*John F. G. Stokes, sine numero*, foot of plateau, southeast, Niihau, January 1912 (Herb. Bishop Mus., type).

Bidens asplenioides, sp. nov. (pl. XII).—Supra herbacea, infra verisimiliter suffruticosa, glabra, ramosa, caule subtetragono, ±4 dm. alto. Folia submembranacea, pinnata aut ternata, crenata, petiolis adjectis 6–16 cm. longa; foliolis lanceolatis aut anguste ovato-lanceolatis, non ciliatis, terminali ad apicem longe acuminato, 6–8 cm. longo, lateralibus ad apicem acutis vel subobtusis et dimidio brevioribus; petiolis tenuibus 3–7 cm. longis. Capitula multa, ligulata, ad anthesin circ. 1.5–2 cm. lata et 6–8 mm. alta, pedunculis tenuibus 1–6 cm. longis. Involucri bracteae exteriores circ. 5, lineari-spathulatae, demum reflexae, glabratae, circ. 2 mm. longae; interioribus lanceolatae, dimidio longioribus. Ligulae (mancas tantum vidi) flavae, circ. 8–10 mm. longae.

Achaenia (manca vidi) linearia, exalata, supra glabrata aut sparsim setosa, apice nuda aut biaristata, verisimiliter 5–7 mm. longa.

SPECIMENS EXAMINED.—*J. F. G. Stokes*, Kaali, Niihau, January 1912 (Herb. Bishop Mus., type).

The elongate crenate terminal leaflets offer a curious superficial resemblance in outline to the leaves or leaflets of some species of *Asplenium* (*A. pinnatifidum* Nutt. etc.). In shape of leaves, length of petioles, habit of inflorescence, number of capitula, proportionate length of exterior involucral bracts, and various other respects, this species is sharply separate from *B. Stokesii*.

Bidens valida, sp. nov.—Supra herbacea, infra verisimiliter fruticosa, glabra, caule tetragono, valido, ±7 dm. alto. Folia (exsiccata) atra supra, acriter serrata, non ciliata, petiolis adjectis 4–15 cm. longa; superiora indivisa ovata aut ovato-lanceolata, abrupte acuminata, 2–6 cm. lata; inferiora tripartita (aut interdum pinnata?—tantum unum inferius vidi), foliolis lanceolatis, acuminatis petiolis tenuibus 1–5 cm. longis. Capitula pauca, corymbosa solitaria in pedunculis subtenuibus, maiuscula, involucro ad anthesin circ. 6 mm. alto et (supra) 11 mm. lato, demum circ. 1.4 cm. alto et (supra) 1.2–3 cm. lato; pedunculis saepe bracteatis, 2–11 cm. longis. Involucri bracteae exteriores 7 aut 8, foliosae, obtuse oblongo-lanceolatae, glabrae, apice obscure induratae, demum 1.5–1.8 cm. longae et 2–3 mm. latae, interioribus longiores. Ligulae non observatae. Achaenia linearia, nigra, exalata, glabra aut sparsim setoso-hispida, apice vero exaristata, plerumque sub apicem biaristata aristis brevibus et retrorsum (1–3 setis) hispidis, 8–13 mm. longa.

SPECIMENS EXAMINED.—*C. N. Forbes* 27 K, Haupu near Lihue, Kauai, July 9, 1909 (Herb. Bishop Mus., type; Herb. Field Mus., no. 485137).

Bidens cuneata, sp. nov. (pl. XIII).—Frutex ramosus, verisimiliter 6–10 dm. altus, ramis dichotomis, tenuibus, infra foliosis, supra in pedunculos productis. Folia crassiuscula, rhomboideo-ovata, dentata (dentibus in latere singulo plerumque 3–5), ad apicem acuta, ad basim anguste aut late cuneata, petiolis adjectis 3–5 cm. longis et 1–2 cm. latis, petiolis tenuibus, 1–2 cm. longis. Capitula solitaria, ligulata, ad anthesin circ. 6 mm. alta et 2–2.5 cm. lata, pedunculis tenuibus 0.8–1.8 dm. longis. Involucri bracteae exteriores circ. 7, lineares, glabratae, glandulo-apiculatae, bracteas interiores subaequantes. Ligulae late lanceolatae, flavae, ad

apicem dentulatae, 8–11 mm. longae. Achaenia linearia, exalata, ad margines sparsissime ciliata, ad apicem ciliato-coronata, exaristata, 6–7 mm. longa.

SPECIMENS EXAMINED.—*W. A. Bryan*, Diamond Head, Oahu, in 1903 (Herb. Bishop Mus., type).

Bidens setosa, sp. nov.—Gracilis, glabra, supra herbacea, infra forsan suffruticosa, caule tetragono, ramoso, ±8 dm. alto. Folia membranacea, plus minusve ciliata, saepe sparsissime adpresso-hispida, serrata dentibus mucronatis; summa nunc indivisa, ovata aut lanceolata, ad apicem acuta vel acuminata, petiolis adjectis 3–6 cm. longa, nunc tripartita; inferiora tripartita vel pinnata foliolis ovatis vel lanceolatis, petiolis adjectis 4–7 cm. longa et 2.5–3.5 cm. lata; petiolis tenuissimis, 1–3 cm. longis. Capitula corymboso-paniculata interdum numerosa, parva, ligulata, ad anthesin 5–6 mm. alta et 1.5–2 cm. lata, pedunculis tenerrimis 1–4 cm. longis. Involucri bracteae exteriores 4–6, patentes aut reflexae, lineares, ciliatae et plus minusve pubescentes, 1.5–2.5 mm. longae, interioribus breviores. Ligulae plerumque 5, flavidae, anguste oblongo-ellipticae, apice (saepe profunde et acriter) dentatae, 5.5–8 mm. longae. Achaenia lineari-fusiformia, interiora supra anguste elongata, omnia exalata, exaristata, plerumque valde setoso-hispida setis singulis aut saepe 2–5-aggregatis, apice setoso-coronata, 7–10 mm. longa.

SPECIMENS EXAMINED.—*C. N. Forbes* 811 K, Waimea Drainage Basin, west side, Kauai, July 3 to August 18, 1917 (Herb. Bishop Mus., two type sheets; Herb. Field Mus., no. 485165).

Bidens Forbesii, sp. nov. (pl. XIV).—Herbacea supra, infra verisimiliter fruticosa, caule ramisque tetragonis, glabra, probabiliter 7–10 dm. alta. Folia inferiora magna, tripartita, petiolis adjectis 1–2.5 dm. longa et 5–15 cm. lata, foliolis lanceolatis, longe acuminatis, membranaceis, creberrime serratis dentibus acribus et longe mucronulato-inflexis, 1–1.4 dm. longis et 3.5–5 cm. latis, petiolis tenuibus 6–8 cm. longis; foliis superis minoribus, 7–10 cm. longis et 4–5 cm. latis. Capitula parva, supra folia exserta, subcorymbosa, ad anthesin 4–5 mm. alta et circ. 1.5 cm. lata. Involucri bracteae exteriores circ. 3–4, anguste lineares, ad apicem acutae, glanduloso-pulverulentae aut fere glabratae, patentes aut

reflexae, circ. 1.5 mm. longae, interioribus paulo longioribus. Ligulae circ. 5, flavidae, anguste oblongo-obovatae, apice valde acriterque 2-dentatae, 6–8 mm. longae. Unum achaenium maturum visum nigrum, valde arcuatum et tortum, glabrum, exalatum, exaristatum, circ. 1 cm. longum; achaeniis immaturissimis biaristatis, aristis retrorsum 1–2-hamosis.

SPECIMENS EXAMINED.—*C. N. Forbes* 82 K, Waioli Valley, Kauai, July 23, 1909 (Herb. Bishop Mus., two type sheets).

Bidens waianensis, sp. nov.—Frutex glaber, supra ramosus, verisimiliter 5–8 dm. altus. Folia gracilia, pinnata aut plus minusve bipinnata, petiolis adjectis 4–12 cm. longa et 3–6 cm. lata, foliolis primariis lanceolatis serratis acuminatis aut iterum pinnatis lobis ultimis linearibus integris ad apicem acriter mucronatis, petiolis tenuissime 2–4 cm. longis. Capitula multa, corymbosa aut corymboso-paniculata, ad anthesin circ. 6 mm. alta et 1.5–2 cm. lata, breviter supra folia exserta, floribus 15–25. Involucri bracteae exteriores circ. 6, lineares, glabratae aut sparsim glanduloso-pulverulentae, ad apicem subacutae, 1–2 mm. longae, quam bracteae interiores dimidio breviores. Ligulae circ. 5, flavidae, oblongo-oblanceolatae, ad apicem obtusae, circ. 1 cm. longae. Achaenia nigra, valde torta, glabra aut versus apicem remote setosa, exalata matura exaristata et 6–10 mm. longa.

SPECIMENS EXAMINED.—*C. N. Forbes* 2023 O, Kolekole Pass, Waianae Range, Oahu, February 1 and 2, 1915 (Herb. Field Mus., no. 485291, type; Herb. Bishop Mus., a form with leaflets much broader than in the type specimen); U.S. S. Pacif. Expl. Exped. (under *Captain Wilkes*), Kaala Mountains, Waianae Range, Oahu, 1838–1842 (Herb. Bishop Mus.; Herb. New York Bot. Gard., two sheets); *J. F. G. Stokes*, Kolekole Pass, Waianae Range, Oahu, in 1915 (Herb. Bishop Mus.).

ASA GRAY had determined the specimens collected under Captain WILKES as being *Bidens micrantha* Gaud. (*Coreopsis micrantha* Gray). Later, in discussing *Bidens micrantha* (Proc. Amer. Acad. 5:127. 1862), he said: "Sandwich Islands, especially Oahu. Variable in the foliage, which is commonly more dissected than in GAUDICHAUD's figure." Clearly GRAY had in mind the *Wilkes* plants, collected in the Waianae Range on Oahu. A study of the more recent specimens by FORBES and by STOKES, collected in the same immediate locality, shows the identical peculiarities of foliage. Furthermore, the floral and achenial characters are seen to be very distinct from those of the more widely distributed *Bidens micrantha*, which occurs not only on Oahu but also on Hawaii, Maui, and Lanai.

Bidens torta, sp. nov.—Fruticosa, glabra, caule non crasso ±5 dm. alta. Folia tripartita, membranacea, serrata, ciliata, tenerrime petiolata, petiolis adjectis 7–16 cm. longa et 2.5–10 cm. lata, foliolis acuminatis, terminali multo maiore, oblongo-lanceolato, lateralibus sessilibus aut breviter petiolulatis, ovato-lanceolatis, petiolis 1–4 cm. longis. Capitula numerosa, laxe paniculata, mediocria, ligulata, ad anthesin circ. 5 mm. alta et 1.7 mm. lata. Involucri bracteae exteriores circ. 5, tenuiter lineares, glanduloso-pubescentes, 1.5–2.5 mm. longae, interioribus paulo breviores. Ligulae circ. 5, oblongo-oblanceolatae, flavae, ad apicem plus minusve dentulatae, circ. 7 mm. longae. Achaenia tenuiter linearia, nigra, maxime torta, glabra, corpore 9–13 mm. longa ad apicem calva aut obscure 1–2-aristata aristis glabris brevissimis (0.1–0.3 mm. longis).

SPECIMENS EXAMINED.—*C. N. Forbes* 2092 O, Kawailoa, Oahu, March 2–5, 1915 (Herb. Bishop Mus., type; Herb. Field Mus., no. 485294).

The leaves of this species appear to have rather large leaflets in proportion to the thickness of the petiole. The terminal leaflet becomes 1 dm. long and 4.4 cm. wide. The branches of the inflorescence are slender and widely diverging. The leaves and inflorescence combine to give a striking superficial resemblance to certain Central American specimens of *B. squarrosa* H. B. K. The achenes surpass those of nearly all other species in the amount of twisting. The twisting commences early, in the young achene, and the mature achenes are frequently twisted through four or five complete revolutions.

BIDENS GRACILIS Nutt., Trans. Amer. Phil. Soc. Ser. II. **7**:368. 1841; *Campylotheca gracilis* Walp., Rept. Bot. Syst. **2**:618. 1843.—No described species of *Bidens* has been left heretofore in greater obscurity than *B. gracilis*. From the time of NUTTALL'S original description, no botanist appears to have given it serious attention. In 1843 WALPER categorically transferred the species, along with two others described by NUTTALL, to *Campylotheca*. In 1862 GRAY (Proc. Amer. Acad. **5**:128) referred it, along with *Bidens mutica* Nutt., to *B. sandvicensis* Less. NUTTALL'S types of *B. gracilis* and *B. mutica* are still extant in a state of excellent preservation (Herb. Brit. Mus.). The type of *B. gracilis* is clearly distinct from that of *B. mutica*. It is distinct also from the specimens that I assume to be of the type collection of *B. sandvicensis* Less. by CHAMISSO from Oahu (for example, *distrib. Acad. Petropol. in Hb. Kew, ex Hb. Hookeri*). In 1888 HILLEBRAND doubtfully referred

the species to a variety of *Bidens macrocarpa*, but NUTTALL's type is not even remotely matched by the type material (Herb. U.S. Nat. Mus.; Herb. N.Y. Bot. Gard.; Herb. Gray) of *B. macrocarpa*. It is, however, the same as *Mann* and *Brigham* 98, distributed to various herbaria as *B. hawaiensis*. *B. hawaiensis* is a much coarser plant and differs in many characters from *B. gracilis*. From all other species of the Hawaiian Islands *B. gracilis* is sharply distinct. From the several specimens studied, I have drawn up the following amplified description:

BIDENS GRACILIS Nutt., descript. amplificat. (pl. XIII).—Frutex gracilis, glabra, ramosa ramis rubescentibus, verisimiliter 6–9 dm. alta. Folia plerumque serrata aut etiam laciniato-dentata, acuminata; nunc indivisa et ovata aut lanceolata, petiolis adjectis 3–7.5 cm. longa et 1–2 cm. lata; nunc tripartita, foliolis lanceolatis, foliolo terminali 4–5 cm. longo et 1–1.5 cm. lato, lateralibus dimidio minora; petiolis tenuibus, 1–2 cm. longis. Capitula parva, paniculata paniculis trichotomis, ligulata, ad anthesin 6–7 mm. alta et circ. 1 cm. lata, pedunculis gracilibus 0.5–2.5 cm. longis. Involucri bracteae exteriores lineares, patulae, supra subglandulosae, interioribus adpressis fere dimidio breviores. Ligulae circ. 5, lanceolatae, flavae, ad apicem dentatae, 3–6 mm. longae. Achaenia torta, linearia, corpore ± 8 mm. longa, facie et marginibus glabra aut sparsissime hispida, apice setuloso-ciliata, nunc brevissime biaristata aristis 0.3–0.8 mm. longis et glabris aut versus apicem retrorsum hispidulis, nunc uniaristata aut etiam exaristata, saepe omnibus tribus formis in eodem capitulo.

SPECIMENS EXAMINED.—*Thos. Nuttall*, Oahu (Herb. Brit. Mus., type); *Mann* and *Brigham* 98, Oahu (Herb. Bishop Mus.; Herb. Cornell Univ.; Herb. Field Mus.); *C. N. Forbes* 1184 O, Moanalua Valley, Oahu, March 9, 1909 (Herb. Bishop Mus.).

BIDENS MICRANTHA Gaud. Voy. Freycinet Bot. 464. *pl. 85.* 1826–1829.—*Campylotheca micrantha* Cass. Dict. Sci. **51**:475. 1827; *Coreopsis micrantha* Gray, Proc. Amer. Acad. **5**:127. 1861; *Bidens Remyi* Drake del Cast. Illustr. Fl. Ins. Mar. Pacif. *pl. 39.* 1888; *Coreopsis Remyi* Drake del Cast., *loc. cit.*, 210.—The identity of *Bidens micrantha* Gaud. has long been a matter of conjecture with most authors. Many appear to have assumed that GAUDI-

CHAUD'S original plate was only a crude representation, and that hence the delineation of foliage, etc., given there must not be interpreted very literally. Consequently, various other species have been referred arbitrarily to *B. micrantha* to such an extent that references in literature to *B. micrantha* Gaud. are almost entirely untrustworthy. In studying the recent collections from the Hawaiian Islands, I was impressed with the resemblance of a certain plant (*G. C. Munro* 602, see later) to GAUDICHAUD'S illustration. The leaves possessed the same peculiar outlines as in the drawing. A careful study of the plant showed that it was positively the true *B. micrantha*. Several other plants that, while varying in various minor details from this plant, were seen to belong nevertheless with it specifically, were then assembled. From this small group of specimens I have been able to draw up the following amplified description.

BIDENS MICRANTHA Gaud., descript. amplificat.—Frutex glabra, caule plus minusve rubido, 6–9 dm. alta. Folia gracilia, crassiuscula, irregulariter 3–5-foliolata aut summa simplicia, petiolis adjectis 4–13 cm. longis, foliolis anguste lanceolatis, acuminatis, utroque latere paucis dentulis ad medium serrato, 2–5 cm. longis et 4–12 mm. latis, petiolis 1.5–5 cm. longis. Capitula numerosa, paniculata aut corymbosa, ligulata, ad anthesin 4–6 mm. alta et 1.5–2 cm. lata, pedicellis tenerrimis 1–2.5 cm. longis. Involucri bracteae exteriores lineares, resino-pubescentes aut glabratae, minimae (circ. 1.5 mm. longae), bracteis interioribus multo minora. Ligulae 3–5, anguste oblongae, flavae, saepe ad apicem obscure dentatae, circ. 1 cm. longae. Achaenia linearia, nigra, compressa, recta vel torta, facie et marginibus plerumque glabra, 7–10 mm. longa, apice nunc exaristata et setosa, nunc breviter biaristata aut etiam (marginibus excurrentibus sub apicem) irregulariter quadriaristata, aristis glabris aut retrorsum hispidulis.

SPECIMENS EXAMINED.—*M. J. Remy* 281, Hawaii, 1851–1855 (Herb. Gray; Herb. Mus. Hist. Nat. Paris; type material of *Bidens Remyi* Drake del Cast. *non nobis; Coreopsis Remyi* Drake del Cast.); *C. N. Forbes* 14 H., Puuwaawaa, Hawaii, June 8–14, 1911 (Herb. Bishop Mus.); *idem* 326 Mo., ridge and foot of Lahainaluna Valley, Maui, February 1913 (Herb. Bishop Mus.); *G. C. Munro* 602, ridge to Puu Kukui, Maui, September 26, 1916 (Herb. Bishop Mus.; one of the achenes was 3-awned!); *idem* 122, Waiapaa, Lanai, September 26, 1913

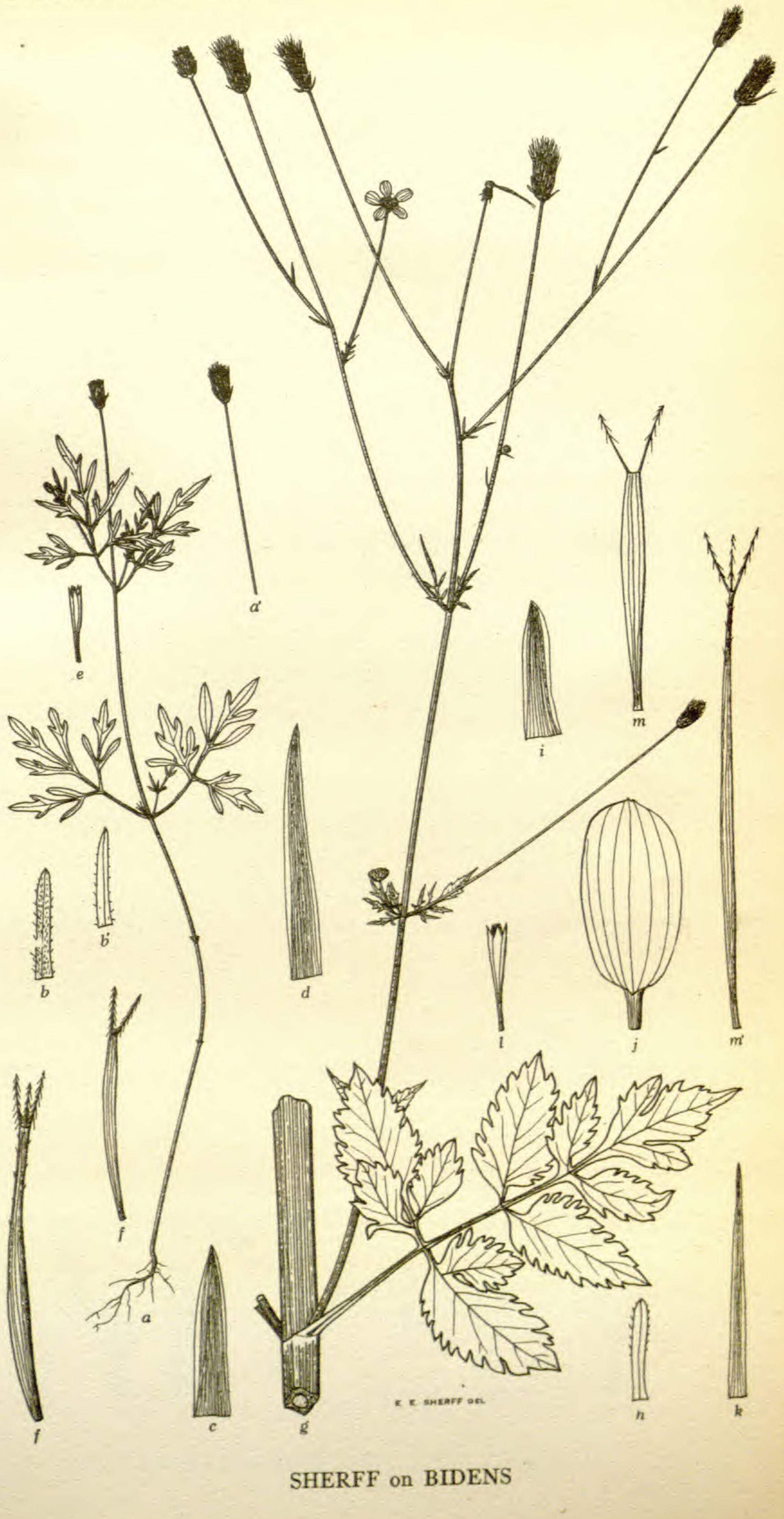
(Herb. Bishop Mus.; form very close to GAUDICHAUD's original plate); *C. N. Forbes*, Kaala Mountains, Makaha Valley, Oahu, February 12–17, 1909 (Herb. Bishop Mus.; Herb. Field Mus., no. 485330; a form somewhat atypic as to foliage); *Hillebrand* and *Lydgate*, Kula, Maui (Herb. Bishop Mus.).

BIDENS MACROCARPA (Gray) Sherff.—*Coreopsis* (*Campylotheca*) macrocarpa Gray, Proc. Amer. Acad. **5**:126. 1862.

BIDENS MACROCARPA, descript. amplificat.—Fruticosa, erecta, glabra (1–1.6 m. alta fide Hillebr. Fl. Haw. 214. 1888). Folia subcrassa, ternata aut pinnata aut summa saepe maximam partem simplicia, petiolo adjecto 0.5–2.2 dm. longa; foliolis (3–5) ovatis aut ovato-lanceolatis, cuspidatis, acriter et saepe creberrime serratis (dentibus interdum valde inflexis), lateralibus 2–6 cm. longis et 1–2 cm. latis, terminali maiore et saepius acuminato, petiolulis lateralium plerumque 2–15 mm. longis; petiolis tenuibus, 2–10 cm. longis. Inflorescentia laxa, aperta, foliolis linearibus vestita, folia superans; capitulis non numerosis, non minutis, ligulatis, ad anthesin 7–8 mm. altis et circ. 3 cm. latis. Involucri bracteae subaequales, exteriores (5–7) crassae, late lineares, glabrae, circ. 6 mm. longae. Ligulae (5–7) flavae, trifida, 1–1.6 cm. longae; disci floribus 15–20. Achaenia pro capitulo magna, late linearia, striata, glaberrima aut marginibus et apice setulosa, erecta aut subtorta, 1.2–2 cm. longa, exalata aut anguste alata, alis in duo dentes aut aristas sub achaeni corporis apicem productis; aristis remotissime et minutissime, antrorsum et retrorsum barbatis, raro glabratis.

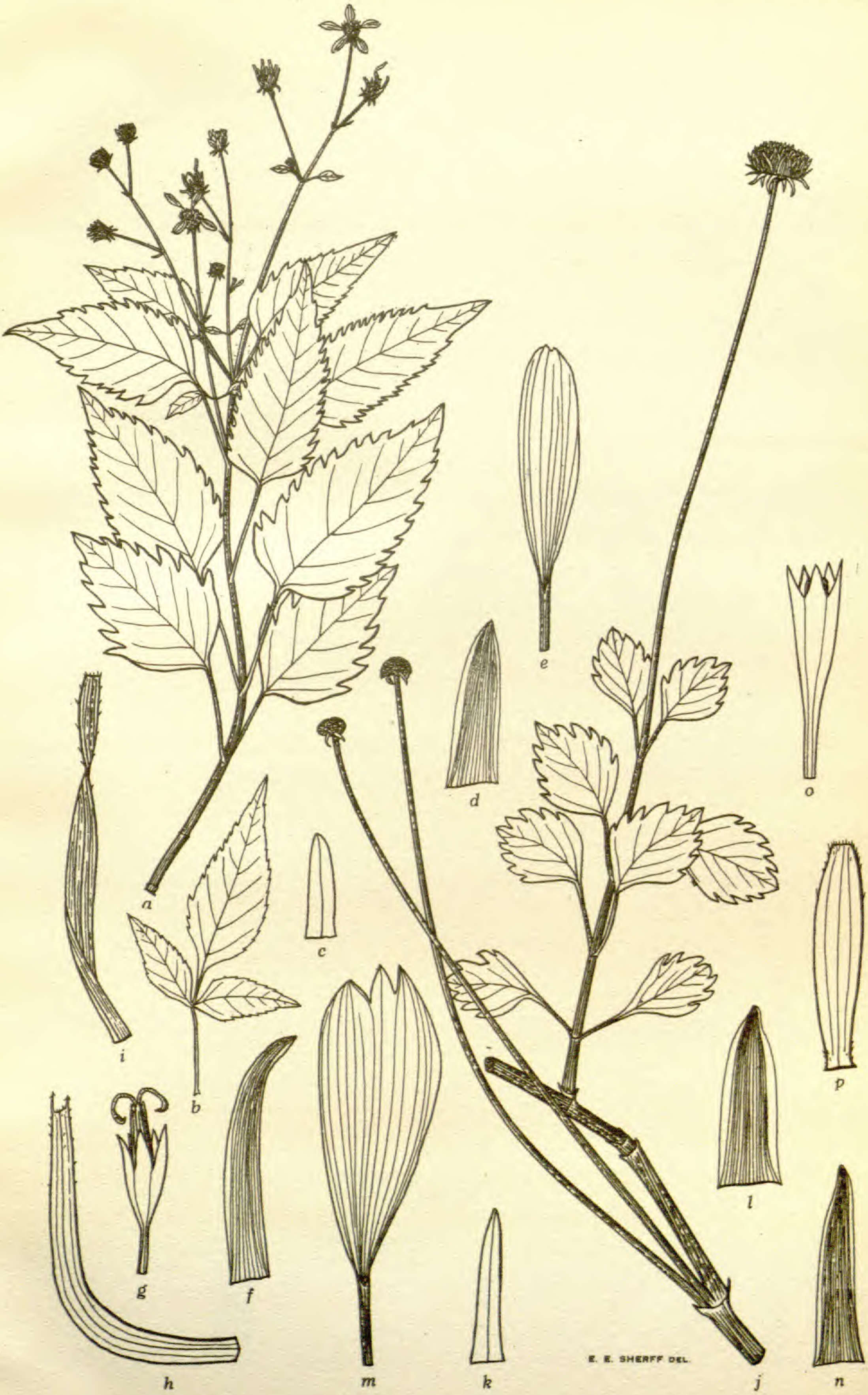
SPECIMENS EXAMINED.—*Capt. Wilkes*, U.S. S. Pacif. Expl. Exped., 1838–1842, Oahu (Herb. U.S. Nat. Mus., type; Herb. N.Y. Bot. Gard.; Herb. Gray); *Hillebrand* and *Lydgate*, Konahuanui, Oahu, Hawaiian Islands, October 1872 (Herb. Bishop Mus.); *A. A. Heller* 2901, on and near the summit of Konahuanui, Oahu, November 2, 1895 (Herb. Mo. Bot. Gard.; Herb. N.Y. Bot. Gard.; Herb. U.S. Nat. Mus.); *C. N. Forbes* 2221 O, Wahiawa-Kahana trail, Oahu, August 17–20, 1915 (Herb. Bishop Mus.); *idem*, Palolo Valley ridges, Oahu, December 17, 1908 (Herb. Bishop Mus.); *idem* 2313 O, ridge and foot, Kalihi Valley, Oahu, March 9, 1916 (Herb. Bishop Mus.); *idem*, Lanihuli Trail, Oahu, October 14, 1908 (Herb. Bishop Mus.); *idem*, Koolauloa Mountains between Punaluu and Kaipapau, Oahu, May 3–8, 1909 (Herb. Bishop Mus.); *idem* 2553 O, Manoa Ridge, Oahu, March 17, 1919 (Herb. Bishop Mus.).

A distinguishing character of this species is the appearance of the large fruiting heads. The achenes become elongate, wide, thickish, and usually very glabrous. In no other species is the tendency to have awns placed below the achene's top (that is, upon the margins and more or less decurrent with the achene edge or wing) more pronounced than here.



SHERFF on BIDENS

SHERFF on BIDENS



SHERFF on BIDENS

SHERFF on BIDENS

Bidens linearifolia (O. and H.), comb. nov.—*Coreopsis linearifolia* Oliver and Hiern, Fl. Afr. Trop. **3**:390. 1877; *Bidens Schweinfurthii* Sherff, BOT. GAZ. **59**:309. 1915.—In 1915, on finding it necessary to transfer this species from *Coreopsis* to *Bidens*, I purposely created the new name *Bidens Schweinfurthii* "to avoid any possible confusion with *Bidens linearifolia* Schz. Bip." (BOT. GAZ. **59**:309). This appeared to me to be much the most desirable procedure. Nevertheless, in view of the fact that *Bidens linearifolia* Schz. Bip. is not a true *Bidens*, but is rather a species of *Cosmos* (*C. linearifolius* Hemsley, Biol. Centr. Amer. **2**:200. 1881), the International Rules require the retention of OLIVER and HIERN'S trivial name.

UNIVERSITY OF CHICAGO

EXPLANATION OF PLATES XI–XIV

PLATE XI

Bidens exigua (*a–f'*) and *B. duranginensis* (*g–m'*): *a*, an entire plant ×0.64; *a'*, fruiting head ×0.64; *b*, *b'*, exterior involucral bracts ×5; *c*, interior involucral bract ×5; *d*, palea ×5; *e*, disk floret ×5; *f*, *f'*, outer and inner achenes ×5; *g*, branch and portion of main stem ×0.64; *h*, exterior involucral bract ×5; *i*, interior involucral bract ×5; *j*, ligulate floret ×5; *k*, palea ×5; *l*, disk floret ×5; *m*, *m'*, outer and inner achenes ×5; *a–f'*, from type of *B. exigua* in U.S. Nat. Herb.; *g–m'*, from type of *B. duranginensis* in Herb. Gray.

PLATE XII

Bidens asplenioides (*a–f*) and *B. Stokesii* (*g–o*): *a*, branch ×0.61; *b*, exterior involucral bract ×6; *c*, interior involucral bract ×6; *d*, ovary ×6; *e* and *f*, fragments of mature achenes ×6; *g–i*, branch and additional leaves ×0.61; *j*, exterior involucral bract ×6; *k*, interior involucral bract ×6; *l*, ligulate floret ×6; *m*, palea ×6; *n*, disk floret ×6; *o*, achene ×6; *a–f*, from type of *B. asplenioides*, *g–o*, from type of *B. Stokesii*, both in Herb. Bishop Mus.

PLATE XIII

Bidens gracilis (*a–i*) and *B. cuneata* (*j–p*): *a*, branch ×0.71; *b*, leaf ×0.71; *c*, exterior involucral bract ×7; *d*, interior involucral bract ×7; *e*, ligulate floret ×7; *f*, palea ×7; *g*, disk floret ×7; *h* and *i*, achenes ×7; *j*, branch and two old peduncles, apparently of previous season's growth ×0.71; *k*, exterior involucral bract ×5; *l*, interior involucral bract ×5; *m*, ligulate floret ×5; *n*, palea ×5; *o*, disk floret ×5; *p*, achene ×5; *a*, *c–i*, from *Mann* and *Brigham* 98, Herb. Bishop Mus.; *b*, illustrating trifoliate leaf, from *Forbes* 1184 O. Herb. Bishop Mus.; *j–p*, from type of *B. cuneata*, Herb. Bishop Mus.

PLATE XIV

Bidens Forbesii: *a*, upper portion of plant ×0.61; *b*, leaf from sterile branch ×0.61; *c*, exterior involucral bract ×6; *d*, interior involucral bract ×6; *e*, ligulate floret ×6; *f*, palea ×6; *g*, disk floret ×6; *h*, very young achene ×6; *i*, mature achene ×6; *a*, *c–i*, from first type sheet, *b*, from second type sheet in Herb. Bishop Mus.

STEM ANATOMY OF DIOON SPINULOSUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 270

LaDema M. Langdon

(WITH PLATES XV–XVII AND FOUR FIGURES)

Introduction

Investigations dealing with the minute anatomical structure of the adult cycad stem have become very numerous and more or less thorough for all of the genera and many of the species, with the exception of the great Mexican representative, *Dioon spinulosum*. This unique and interesting species was first but inadequately described by Eichler (6) in 1883, and by Dyer (5) in 1885. The first extensive account of its general field characters, size, external structure, and distribution was by Chamberlain (1) in 1909. A later article (2) by the same author gives a full and careful description of the macroscopic structure of adult stems of *Dioon spinulosum*, *D. edule*, *Ceratozamia mexicana*, and *Zamia floridana*, particular attention being given to *D. spinulosum*. Special study is made of the growth rings, reported here for the first time in cycads, and of the medullary bundles which constitute the vascular system of the cones, and which are called cone domes, because of the domelike arrangement of these strands with the peduncle of the cone at their apex. The histological characters of the trunk, its growth rings, the thick-walled fibers of the phloem, and the structure of the xylem elements the author considers remarkably similar to the corresponding structures of *Cycadeoidea*.

The embryo and seedling of *D. spinulosum* have been studied recently by Sister Helen Angela (4), and found in the arrangement and orientation of the vascular strands in the cotyledons, hypocotyl, stem, and leaves to differ in no marked degree from the usual cycad arrangement. Features particularly worthy of note in connection with the girdling habit, as this investigator has traced it from macerated seedling stems, is that each leaf is supplied with five strands arising from cauline bundles situated in different parts

of the stem, and further, that these girdling strands are horizontal from the beginning and continue so throughout their whole extent.

The investigation here described was undertaken with a view to supplementing CHAMBERLAIN'S general account of the histological structure of the adult stem of *D. spinulosum*, especially by a careful study of the broad foliar rays or leaf gaps with their included traces, a phase of cycadean anatomy only slightly touched upon by earlier investigators. As the work has progressed, its scope has been extended to include the general course and organization of the foliar strands in the cortical part of both adult and seedling stems.

Material and methods

The adult wood and abundant material of two- and three-year-old seedlings, which furnish the basis for this study, were secured by CHAMBERLAIN from the Hacienda de Joliet near Tierra Blanca, Mexico. The ten-year-old seedlings were from seeds procured in the same locality but germinated and grown in the botanical greenhouse of the University of Chicago.

Preparation of all material of the adult stem for sectioning was in the main as follows. Narrow, wedge-shaped sections extending radially from pith to cortex were cut from both the upper and lower portions of a trunk 18 ft. in height, care being taken that each included two or more of the large medullary rays. These sections were then cut into blocks about 1 cm. square, some slightly larger, and kept in series.

The various stages involved in the preparation of these blocks for sectioning, namely, demineralization of the woody tissues through the use of hydrofluoric acid, followed by a thorough washing of the material in running water to free it from all traces of the reagent, transference to various grades of alcohol and xylol, and finally imbedding in paraffin, have been discussed in a previous paper (8). Special care had to be taken in imbedding, the best results being obtained when the blocks were carried through the process of infiltration with paraffin from 48 hours to 3 days. After this they could easily be cut with a sliding microtome, and a complete series obtained by removing each section, as cut, from the knife and placing it directly upon the slide. Staining was with

safranin and gentian violet, or safranin and "licht grün," the latter combination proving the more satisfactory.

The greater part of the study of the girdling habit in the seedlings was made from cleared material. Entire sections comprising stem cylinder and cortex, in blocks 0.5×1 inch, were cleared so perfectly that it was possible, with the aid of a strong artificial light, to look through such a section and see the vascular strands clearly outlined in the cortex.

In the case of the two- and three-year-old seedlings, the method followed consisted in severing the long taproot from the stem just below the region of the cotyledonary plate and cutting off the long terminal leaves, leaving only the leaf bases and a small part of the petiole. The scale leaves were then carefully trimmed from two sides of the stem, and one clean longitudinal cut made through the entire stem from apex to base. After the transference of these half-sections to 50 per cent alcohol (each seedling being kept in a separate receptacle), the process was substantially the same as that for the paraffin method, that is, up to the pure xylol stage. At this point the material was subjected to vacuum treatment in order to free the tissues, as far as possible, of any air or gases they might contain. As a final clearing agent a mixture of xylol and carbon disulphide was used; the CS_2, having a higher refractive index, rendered the material more transparent than pure xylol.

Adult stem

With the single exception of the Australian *Macrozamia Hopei*, *Dioon spinulosum* Dyer is the tallest of all cycads, ranging 10–30 ft. in height, with occasional specimens reaching 40 and even 50 ft. The particular specimen from which this study was made was about 18 ft. tall and possibly 100 years old. The width of the woody zone from pith to cortex averages 0.5–0.75 inch in the upper part of the trunk and 3.5–4 inches in the lower trunk.

STRUCTURE OF XYLEM.—The adult stele of *D. spinulosum* is endarch, and its compact woody cylinder consists chiefly of longitudinal tracheidal elements and radial parenchyma. From the pith to the cortical part of the stem the length of the tracheids averages as follows: scalariform metaxylem tracheids 4–4.2 mm.,

first pitted tracheids 5–6.5 mm., tracheids in the vicinity of the cambium 7–9.8 mm.

The protoxylem elements are of the reticulate and scalariform types, and in passing from the metaxylem to the first formed elements of the secondary wood all transitional stages occur in the reduction of the scalariform structure into imperfectly formed, multiseriate, bordered pits.

While the majority of the tracheids of the secondary xylem exhibit on their radial walls the multiseriate type of pitting so characteristic of this wood, many of the tracheidal elements, especially those constituting the secondarily formed wood in the upper trunk, have their radial walls covered with small bordered pits of a very irregular arrangement.

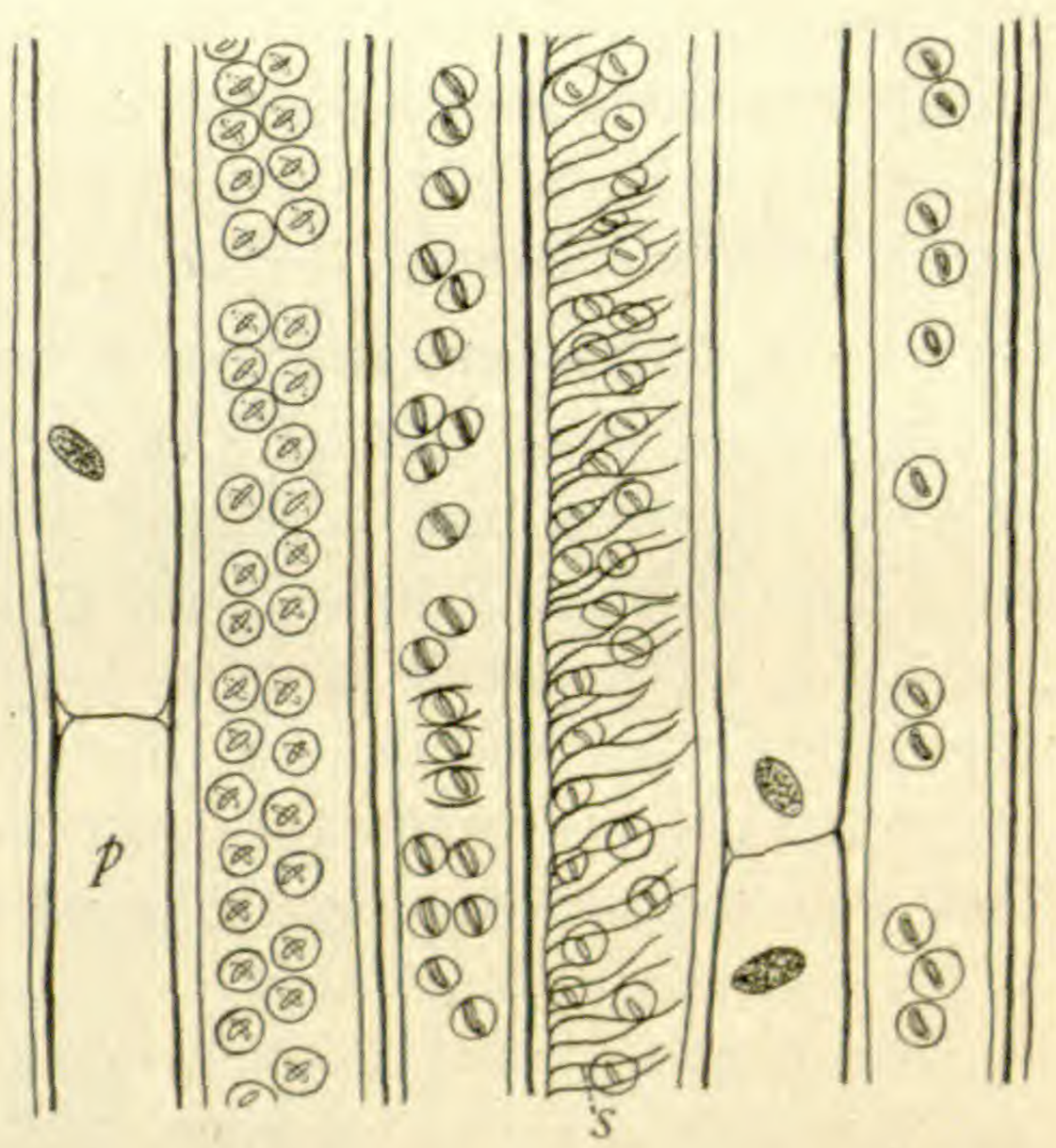


FIG. 1.—Radial longitudinal section of tracheids from lower portion of adult trunk: *s*, tertiary spiral thickenings of tracheid walls; *p*, wood parenchyma; ×225.

In the wood of the lower trunk tertiary spiral thickenings of the tracheid walls were observed occurring in the first few rings of growth and also in the older wood (fig. 1). These spirals are not common to all the tracheids, but are generally sporadic in their appearance and may be quite inconspicuous. In some cases, however, they are characterized by considerable prominence, and are so compact as to suggest a reticulate rather than a spiral formation.

In addition to the lignified elements of the wood there are narrow elongated cells with transverse walls, the longitudinal storage parenchyma. These cells, like those of the radially disposed

bands of parenchyma, are usually well filled with starch and occasional calcium oxalate crystals.

MEDULLARY RAYS.—The medullary rays are of three types, namely, narrow uniseriate rays, a single layer of cells wide and several cells deep; multiseriate rays, two to several cells in width at their widest point and of variable longitudinal extent; and broad foliar rays or leaf gaps, which in tangential view resemble the aggregate ray of *Quercus*. The last are distributed at fairly equal intervals throughout the woody cylinder and always extend from the pith to the cortex. They are further characterized by the presence of at least one mucilage duct and one leaf trace bundle situated in the lower central part of the gap (fig. 6). A few isolated cases occur where two ducts and even two traces may be seen in a single foliar ray.

Course and structure of leaf trace in gap

The course of the leaf trace through the parenchymatous gaps or foliar rays from pith to phloem is approximately level, except for a slight downward curve of the strands due to their manner of formation. The bundle is endarch throughout its course in the gap and through the phloem, the xylem of the bundle usually uppermost and just beneath the duct, with no change in orientation until the bundle reaches the cortex, where it is continuous with one of the oblique cortical strands.

The manner of connection of this foliar trace with the primary and secondary wood of the main stele is one of the most striking and interesting features of the wood. Within a short distance of the pith the strands of the trace curve downward, the primary and secondary elements uniting with like elements of the main cylinder. On the interior vertical face of the wood at the point of union, and continuing up through the gap, always on the upper side of the trace (figs. 3–5) where the primary vessels of the trace would naturally appear, are peculiar tracheidal elements, curiously reticulated, in some cases forming continuous vessels, in other cases merely isolated patches of lignified tissue. These irregular fibrous elements are best illustrated in fig. 2, where a longitudinal section of the upper portion of the trace appears in a transverse section of the wood.

WORSDELL (12) also calls attention to the occurrence of peculiar irregular tracheids resembling "transfusion-tissue" on the interior vertical face of the wood and accompanying the bundles of the large medullary rays of *Macrozamia Fraseri*, and also found among

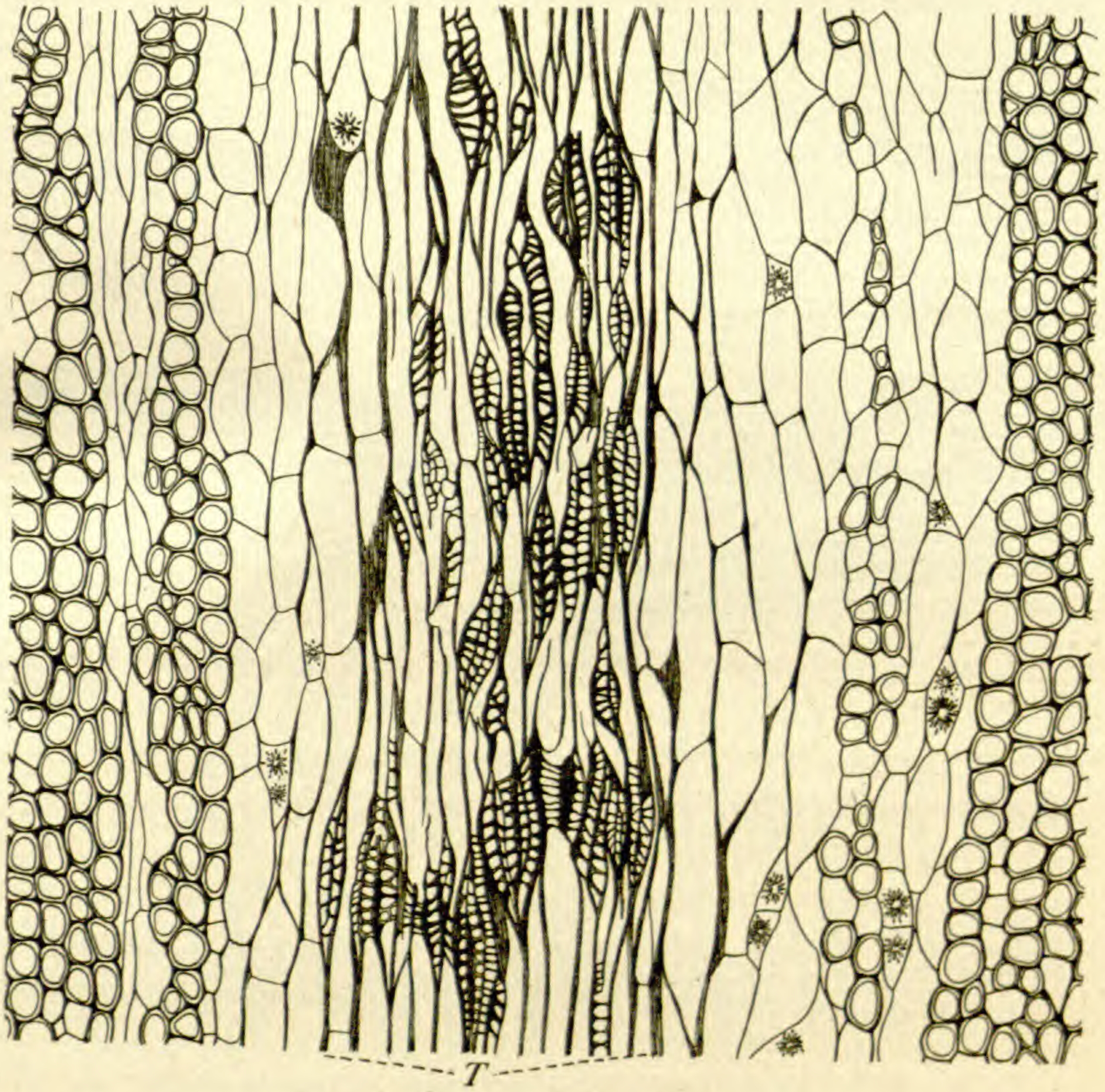


FIG. 2.—Transverse section of mature wood, showing foliar ray in center: *T*, longitudinal section through upper part of leaf trace; ×85.

the parenchyma cells between successive vascular rings in *Encephalartos* (13). These, however, are of a pitted type rather than of the irregularly reticulated and scalariform types characteristic of *D. spinulosum*.

The mode of connection of this trace with the secondary fibrovascular structures of the stem is as follows. Figs. 3, 4, and 5 are

radial longitudinal sections of the large medullary ray with its included foliar strand. A careful study of a series of such radial sections through the trace has made it apparent that this connection is by means of long, irregularly shaped scalariform tracheids which are the real tracheids constituting the trace, and not mere con-

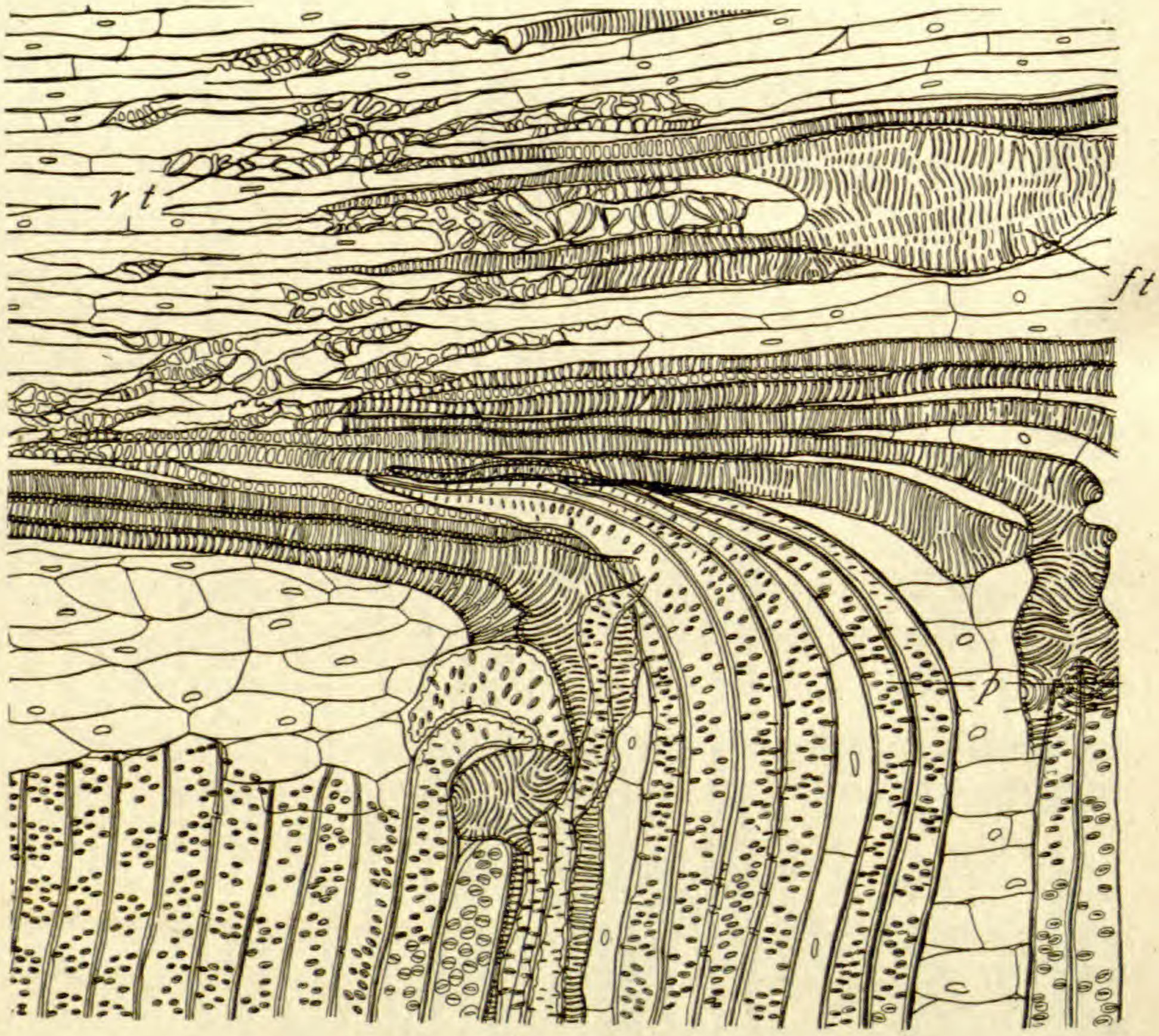


FIG. 3.—Radial longitudinal section of portion of large medullary ray with included foliar strand: *ft*, peculiar tracheidal formation apparently resulting from fusion of two or more tracheids; *rt*, irregularly reticulated elements; ×90.

necting elements, as generally believed. These vessels may be entirely scalariform in structure, or may change gradually from a reticulate type at their tapering ends to decidedly scalariform throughout the greater part of their horizontal and vertical extent. As they extend horizontally through the gap they are arranged in order of formation, one vessel beneath the other (figs. 4, 5), the

lowest and last formed continuing a little farther in the direction of the cortex than the one preceding. In this way they constitute a perfectly continuous conducting system for solutions passing out to the cortical strands. These tracheids are especially numerous a short distance behind the pith, just beyond the primary xylem, but passing on through the gap they appear to become grouped (fig. 5), occurring at intervals with great masses of phloem and parenchyma separating the basal parts of each group. Whether this grouping is associated with a seasonal increase in the length of the trace, corresponding to the radial increase and growth rings of the main stele, has not been determined. In addition to the reticulate and scalariform elements constituting these traces, the regular pitted tracheids of the secondary xylem, usually at the extreme lateral borders of the gap, are often diverted to one side into a direction more or less parallel to that of the trace, as shown in figs. 3 and 5.

WORSDELL (12) has described in the case of *Macrozamia Fraseri* a somewhat similar connection between the fibrous strands in the large medullary rays and the fibrovascular elements of the main stele. He states that "a characteristic feature of the radial section of the wood is the large number of outbending strands of tracheids which, passing through the medullary rays, are continuous with the girdle leaf trace of the cortex." It is worthy of note, however, that these strands of outbending tracheids, or rather inbending in the sense that they are apparently diverted from the direction of the other secondary xylem elements toward that of the rays, are of the pitted type throughout their length, thus homologous with the pitted elements described in the preceding paragraph.

The peculiar down-curving growth of the scalariform tracheids constituting the foliar strands in the large medullary rays of *D. spinulosum* is another interesting illustration of the much discussed phenomenon of gliding growth. Vertically these conducting elements may extend merely to the lower borders of the gap and terminate in the irregular bulbous formations illustrated in fig. 4*B*, or they may become inserted for a considerable depth between the perpendicular fibrous elements of the main stele (fig. 5*sc*). In their horizontal extent these tracheids may be and probably are

the product of cambial activity, but in their vertical enlargement and elongation it seems probable that these lignified elements have simply been stretched out into their curious bending shapes by the growth of the adjoining living parenchyma tissue. The close scalariform markings on these vessels, in some cases approaching almost a pitted character, would indicate that this growth or elongation has taken place gradually, keeping pace with the longitudinal expansion of the gap.

It is also evident that the basal portions of many of these vessels have their origin in quite a different manner. The character of the pitting indicates that there has been a gradual lignification of the ordinary parenchyma cells (fig. 4 *B*), and a subsequent fusion by the breaking down and reabsorption of the partition walls. The formation of the peculiar curved and bulbous-like bases of many of these tracheids, where they come in contact with the perpendicular elements of the secondary wood, is shown in this way.

Course of leaf traces in cortex

The course of the fibrovascular bundles in the cortex, complicated by the well known habit of girdling, was first described by KARSTEN (7) in *Zamia muricata*, in 1856, later, in 1861, by METTENIUS (10), and in more recent articles quite fully by THIESSEN and Sister HELEN ANGELA in seedlings of *Ceratozamia* (3), *Dioon edule* (11), and *D. spinulosum* (4).

A brief statement of the girdling situation in the embryo and seedling of *Dioon edule*, as described by THIESSEN, is approximately as follows. For each leaf or scale leaf there are four distinct strands leaving the vascular cylinder. Two of these leave on the same side as the leaf for which they are destined, and pursue a direct course through the cortex to the central part of the petiole without branching; while the other two strands leave the cylinder approximately on the opposite side, describe a wide curve around it, and finally enter the dorsal part of the leaf petiole, where they branch repeatedly.

Sister HELEN ANGELA (4) has described a similar situation and arrangement of the cortical traces in the seedlings of *Dioon spinulosum*. Both authors agree that there are 4 or 5 strands leaving

the main cylinder for each leaf, each one of these strands describing a separate arc to the point where it enters a leaf base. Furthermore, all girdles are reported as being horizontal throughout their whole extent. It is obvious, therefore, that the phenomenon of girdling, as I have been able to trace it very distinctly and definitely in cleared specimens of two-, three-, and ten-year-old seedlings of *D. spinulosum*, differs in many respects from these earlier accounts. Thus for each leaf or scale leaf 7–9 strands, the number varying with the size of the sheathing leaf base, separate from the vascular cylinder. Two of these (fig. 8 *e*, *e*[1]) leave the cylinder on the same side as the leaf for which they are destined and take an upward, oblique course for some distance, finally passing out more or less directly through the cortex into the ventral part of the petiole.

Two other traces (fig. 9 *a*, *a*[1]) leave the main stele at closely approximated points on the side opposite the leaf for which they are destined and pursue an upward, rather oblique course for some distance. Then, curving one in either direction, they take a horizontal course, describing wide arcs through the cortex and sheathing leaf base, finally entering the dorsal or adaxial part of the petiole, where they undergo a complicated system of branching. The rest of the traces destined for this leaf (fig. 9 *b*, *b*[1], *c*, *c*[1], etc.) leave the main stele at intermediate points and assume, like traces (*a*, *a*[1]), an upward, vertical direction, finally anastomosing with the two horizontal strands as they encircle the cortex. It is also noteworthy that each of these lateral oblique traces leaves the stem cylinder at a point slightly higher than the one preceding, so that the entire course of the two girdles is gradually and spirally ascending to the point where they enter the central part of the leaf base. Frequently a single bundle (fig. 8 *a*), separating from the vascular cylinder on the side opposite the leaf for which it is destined, may divide soon after leaving the central cylinder, the two horizontal branches swinging to right and left in wide curves through the cortex and the sheathing base of the leaf, and gradually anastomosing with the rest of the traces destined for that leaf. The character of the branching of these two main strands after entering the adaxial part of the petiole is so clearly illustrated in fig. 10 that any further discussion of this point is unnecessary.

At the very tip of the stem the traces of the youngest leaves ascend in an almost perpendicular direction about the region of the so-called potential vascular tissue to the point where they connect with the horizontal bundles. At this stage (fig. 10) all of the girdling strands lie in substantially the same plane, the pair associated with the youngest leaf describing slightly smaller arcs than those of the older leaves of the same crown. As internal radial growth and the appearance of new leaves crowd the older parts farther and farther away from their original terminal position, however, the lateral foliar traces become less vertical and more oblique. With this radial and longitudinal expansion of the stem is also associated a lengthening of the horizontal girdling strands, and consequently a widening of the intervals and the arcs between each lateral connecting trace.

These leaf traces are always endarch and collateral as they leave the stem cylinder, and also during their passage vertically and horizontally through the cortex to a point well up in the leaf base. They are so orientated that the xylem and phloem are directed toward the inside and the outside of the stem respectively. Transverse and longitudinal views (fig. 12) throw additional light on the organization of these cortical bundles.

COURSE OF LEAF TRACE IN ADULT STEM.—Due to the difficulties involved in following up strands of such size, it is impossible to determine with certainty whether the arrangement of the leaf traces in the adult stem of *D. spinulosum* is the same as that found characteristic of the seedlings. The problem becomes increasingly difficult as the plant reaches an age when the crown comprises numerous developing leaves. From longitudinal and transverse sections of the adult stem, however, it is evident that the same general relation between lateral oblique traces and a horizontal girdling strand is maintained, but it is probable that the girdling is only partial, that traces a and a^1 (fig. 9) would have their origin at points more remote from each other in the adult stem. It is also probable that there is no appreciable increase in the number of traces associated with successive leaves, beyond the number described as supplying the leaves of the ten-year-old seedling (fig. 10).

Discussion

In his description of the girdling habit, METTENIUS (**10**) finds that "in the developmental stage the traces of the youngest leaves

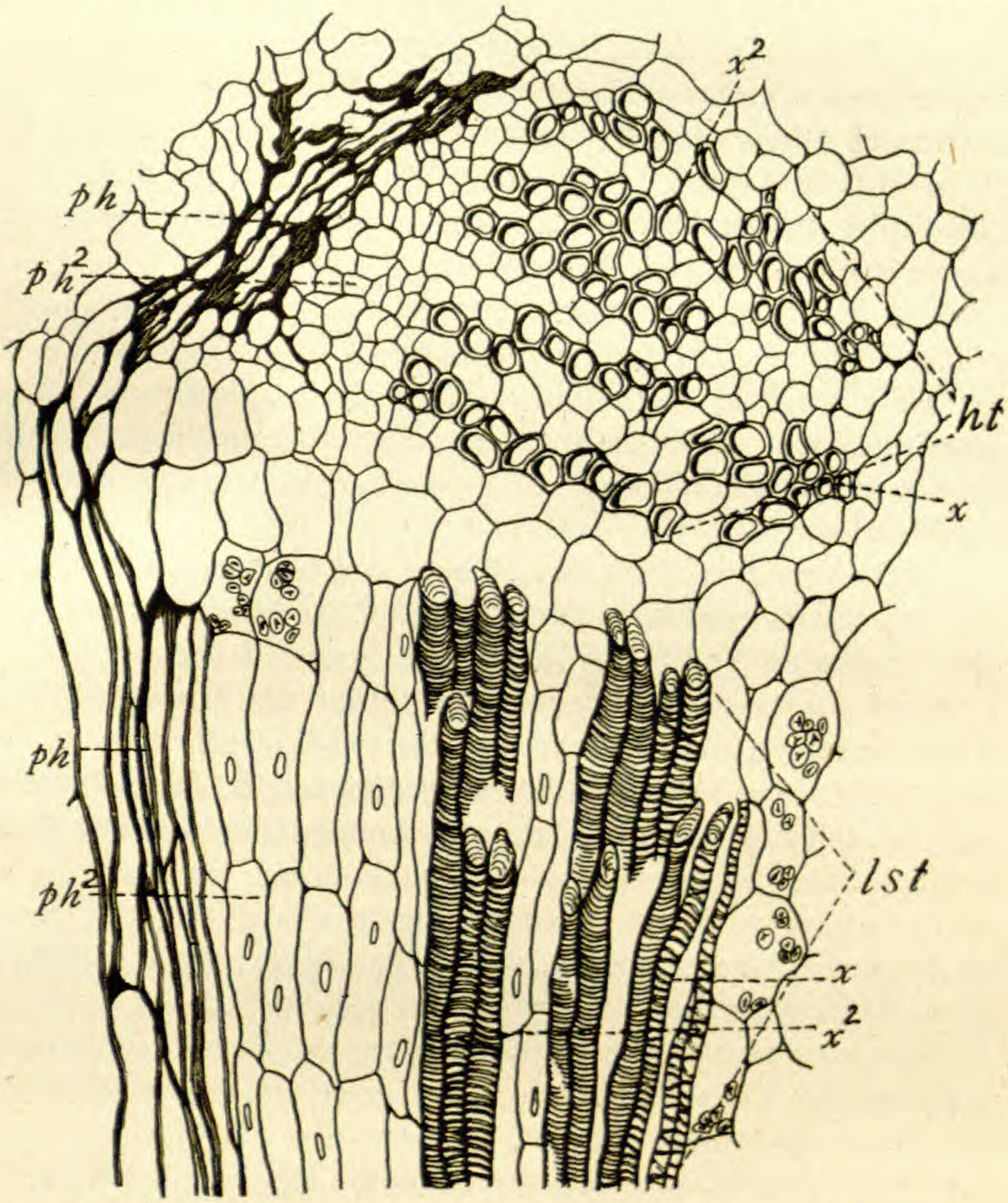


FIG. 12.—Detail of organization of cortical foliar strands of three-year-old plant: *ht*, horizontal strand in transverse section; *lst*, longitudinal section of vertical lateral trace near point of union with horizontal strand; *x*, primary sylem; x^2, secondary xylem; *ph*, primary phloem; ph^2, secondary phloem; ×120.

lie in the region of the vegetative point, and at first ascend in an almost perpendicular direction, but during further growth assume

gradually a horizontal position, and with subsequent growth are lengthened and the expanse increased." MATTE (9) and Sister HELEN ANGELA (3), in connection with *Ceratozamia*, both describe a similar vertical position of traces in the early developmental stages, girdling becoming evident with the increase in diameter of the inclosed group of leaves and stem. In more recent investigations of *Dioon edule* by THIESSEN, and of *D. spinulosum* by Sister HELEN ANGELA (4), however, both authors maintain that the girdles are established very early, and that their horizontal course is laid down from the beginning.

The results of the present investigation indicate two possibilities therefore. Either the arrangement of cortical strands in the older seedlings and adult wood of *Dioon spinulosum* differs from that found in the embryo and very young seedlings of both species of *Dioon*, or the preceding statements need considerable modification.

At the very tips of the two-, three-, and ten-year-old seedlings (fig. 10) the perpendicular arrangement of the lateral strands and their connection with the horizontal girdles is unmistakable. It is reasonable to suppose, therefore, that the arrangement of foliar strands in the first leaves of the young seedling would be substantially the same as that characterizing the leaves of the older seedlings, save that (1) the very young strands having their origin in the cotyledonary plate would ascend vertically for a shorter distance before anastomosing to form the horizontal girdles, and (2) there would be likely to be a decrease in the number of strands leaving the vascular plate for each leaf base.

Another question of importance is the significant relationship suggested by the distribution of leaf traces in the seedlings of *D. spinulosum*. Thus we find numerous strands (varying from 7 to 9) passing obliquely upward into each leaf base, each one of which causes a gap of its own in the main stele. As previously indicated, however, these strands do not all enter the petiole. There is instead an anastomosis of traces in the sheathing base of the leaf, resulting in the conspicuous and characteristic horizontal girdles, which correspond in many respects to the marginal vein of

the sheathing monocotyl leaves, save that the marginal vein of the typically sheathing monocotyl leaf is connected with a large number of bundles which come off around the entire periphery of the stem.

Summary

1. The medullary rays of *Dioon spinulosum* are of three distinct types: uniseriate rays, a single layer of cells wide and several cells deep; multiseriate rays, two to several cells in width and of variable longitudinal extent; and broad foliar rays or leaf gaps, which, with their included leaf traces, are such a constant feature of this wood.

2. The fibrovascular elements constituting the leaf traces in the foliar rays and connecting these traces with the secondary wood are peculiar, irregular scalariform tracheids which in the course of their development curve gradually downward through the ray, until they become inserted between the perpendicular fibrous elements of the main stele.

3. The regular pitted elements of the secondary xylem are also often diverted to one side into a direction parallel to that of the trace.

4. Both the scalariform and the pitted elements constituting these traces, in their peculiar manner of enlargement and elongation, furnish excellent illustrations of gliding growth.

5. For each leaf or scale leaf 7–9 strands (the number varying with the size of the leaf base) separate from the vascular cylinder. The two inner ones, arising from the proximal side of the central cylinder, pursue a more or less direct vertical course into the ventral part of the petiole; the rest of the traces, leaving the stem cylinder at different points, pass obliquely upward into the cortex and the sheathing base of the leaf, where an anastomosis of traces takes place, resulting in the two characteristic girdles.

6. The two direct strands entering the ventral or abaxial part of the leaf may also unite with the two dorsal girdling strands at the base of the petiole, so that the whole system is reducible in the older seedlings and adult stem to two main horizontal strands with their associated lateral traces.

Grateful acknowledgment is made of the helpful criticism and advice given by Professors JOHN M. COULTER, CHARLES J. CHAMBERLAIN, and W. J. G. LAND during the progress of the investigation. The writer is also greatly indebted to Dr. CHAMBERLAIN for the very generous supply of material.

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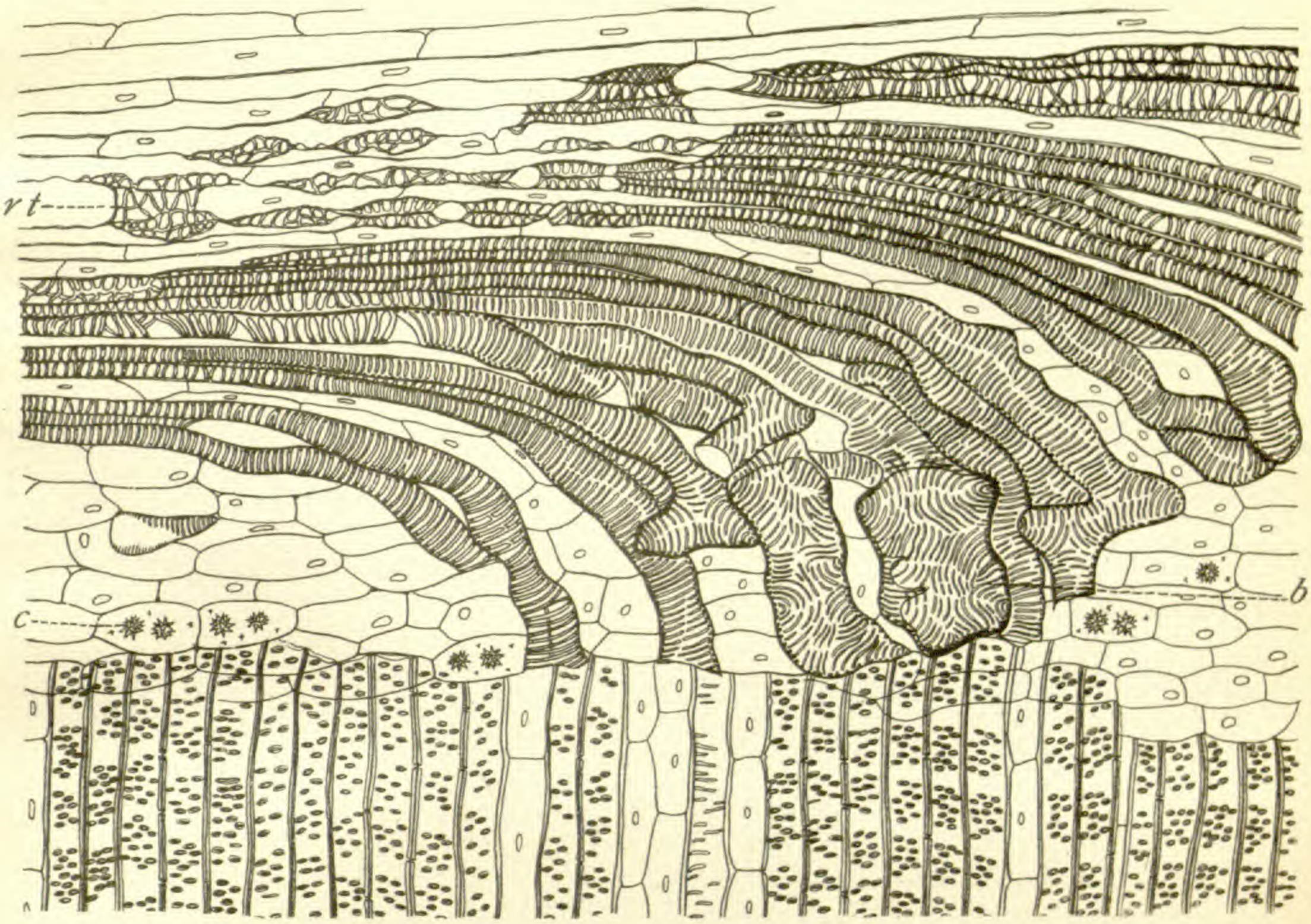
EXPLANATION OF PLATES XV–XVII

All the drawings were made with the aid of a camera lucida, except figs. 8, 9, 10, 11, which are diagrams showing origin and distribution of foliar vascular strands, as traced from cleared material, supplemented, where detail in connection was required, by serial sections; figs. 1–3 and 12 are in the text.

PLATE XV

FIG. 4.—Mature wood: radial longitudinal section of large foliar ray, showing organization of leaf trace and structure of scalariform tracheids of trace: *b*, bulbous bases of tracheids; *c*, calcium oxalate crystals; ×90.



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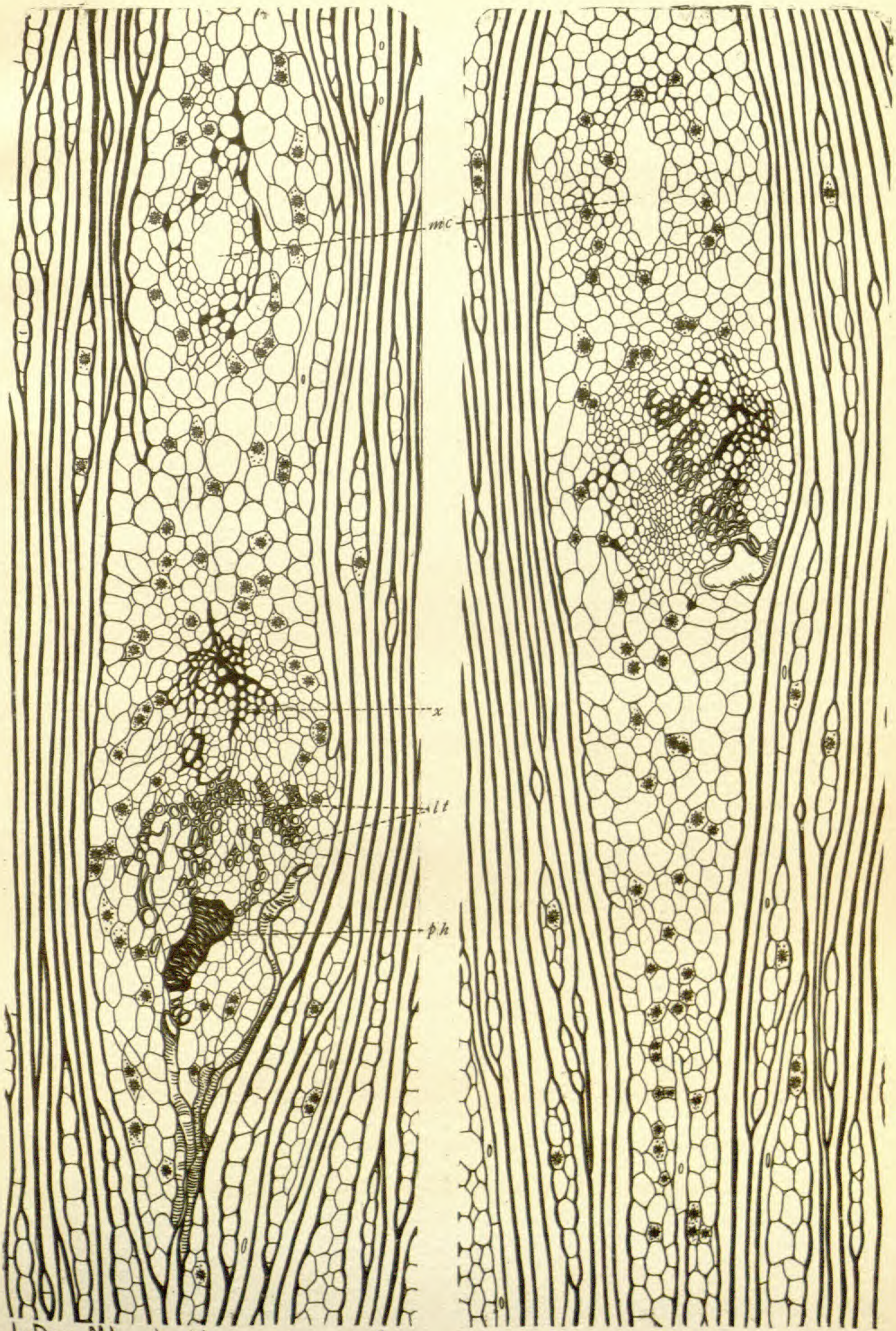

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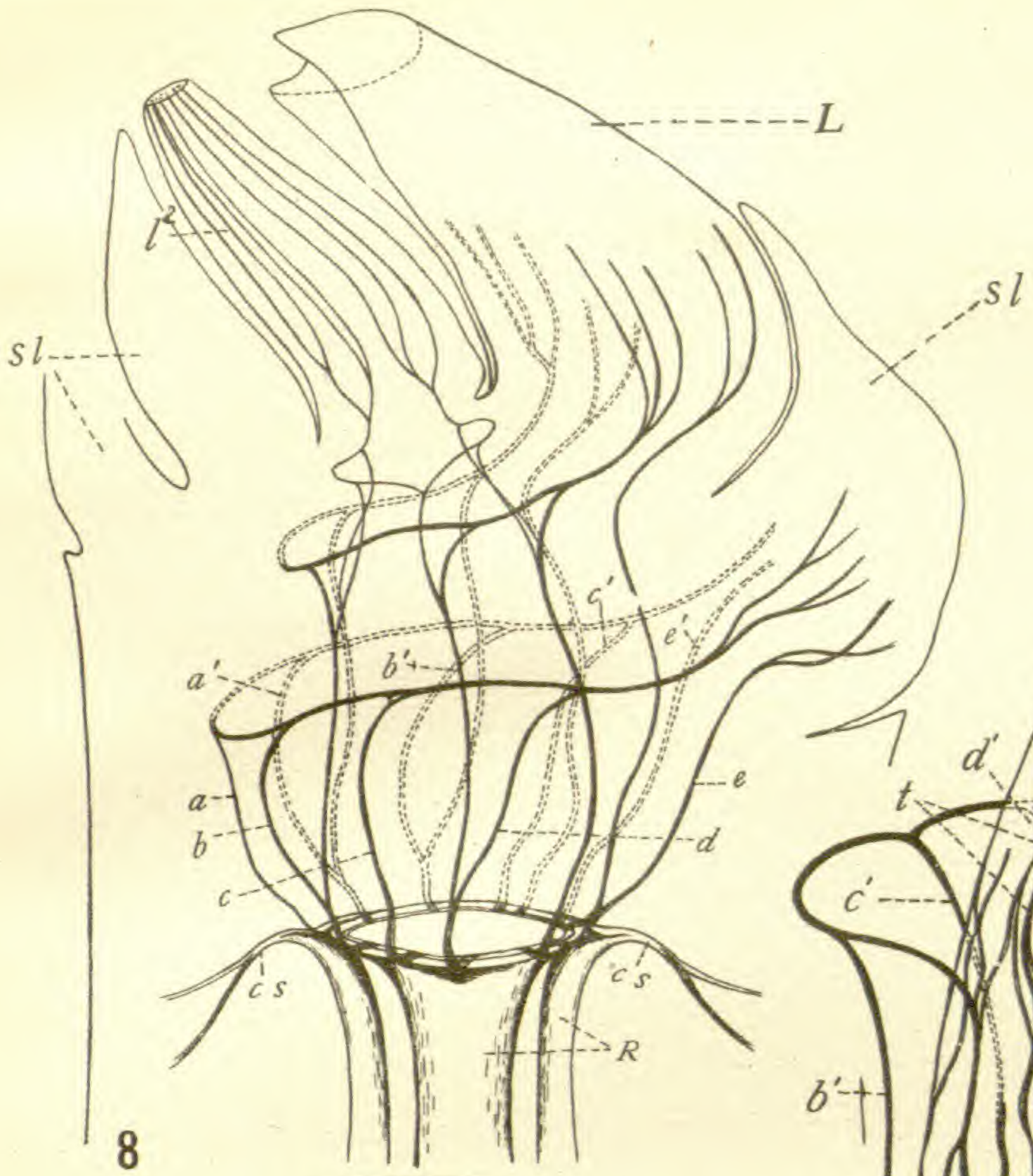

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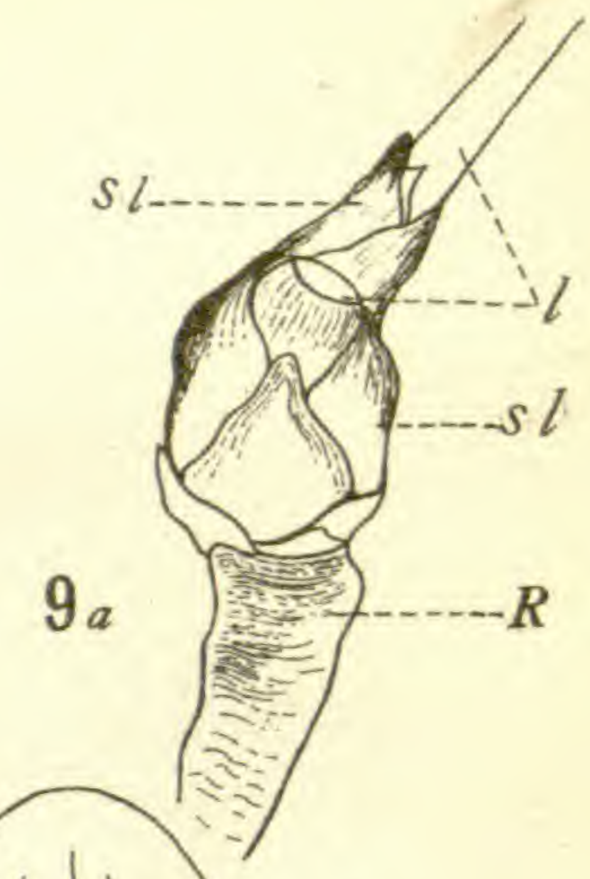

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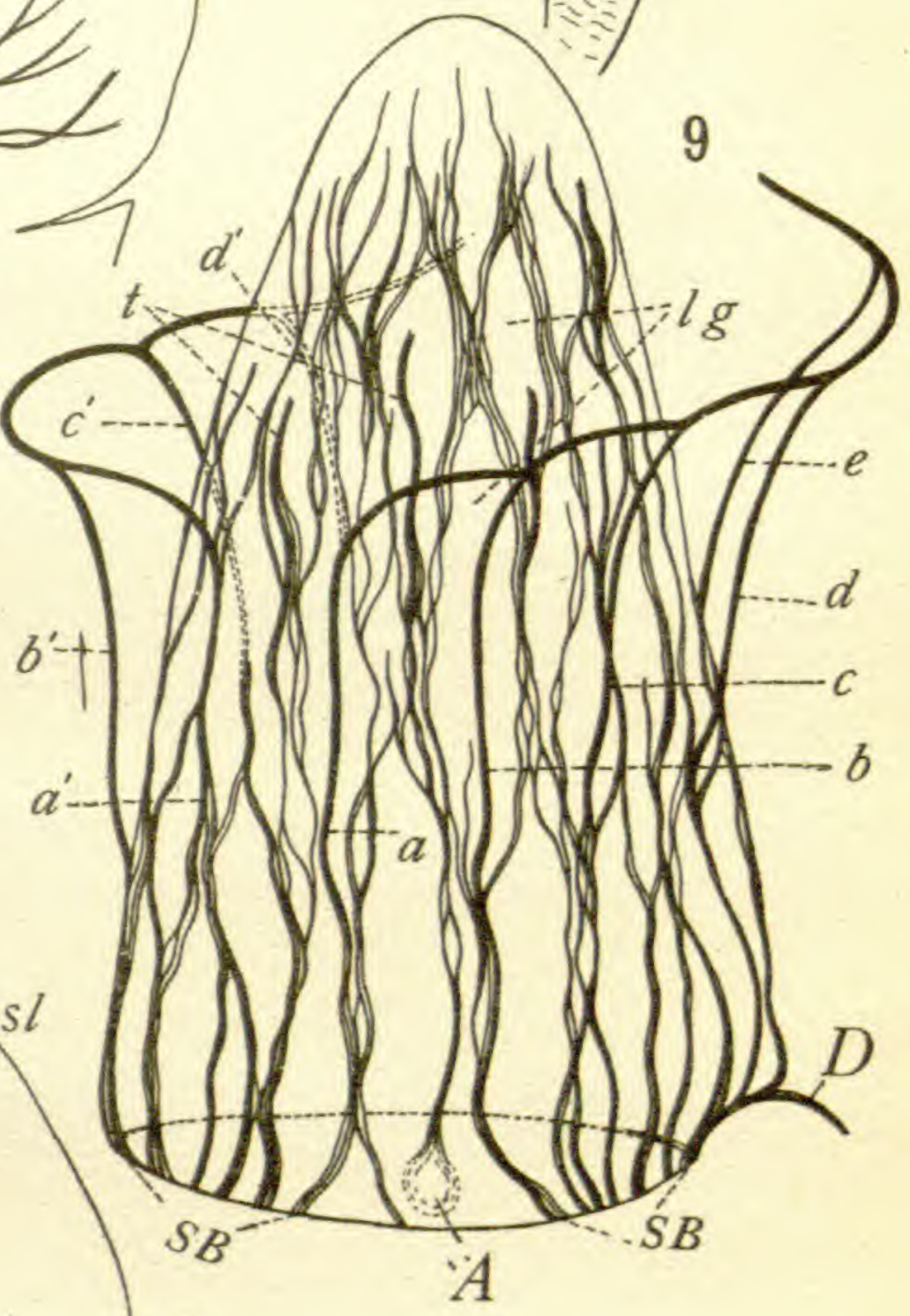

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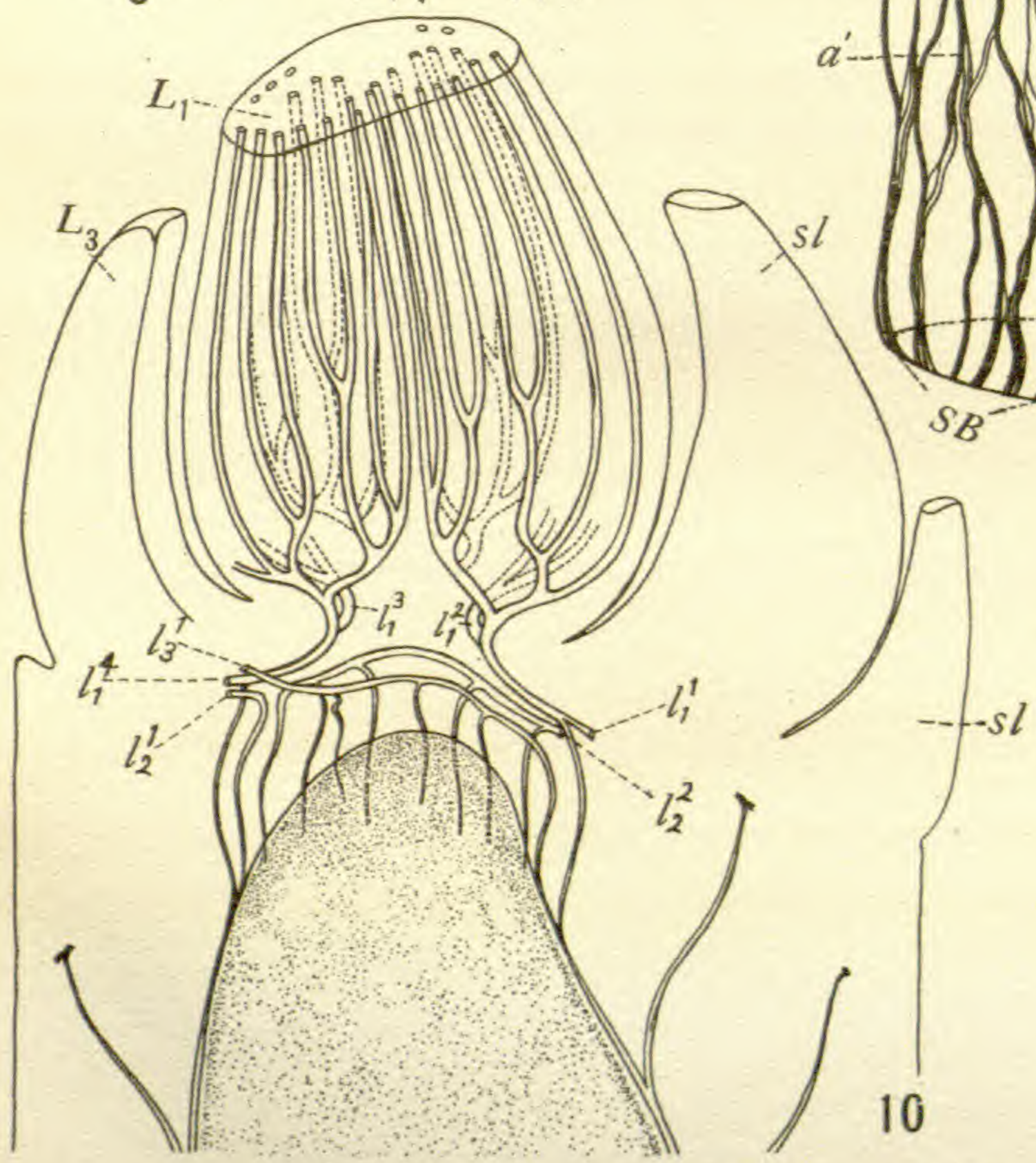

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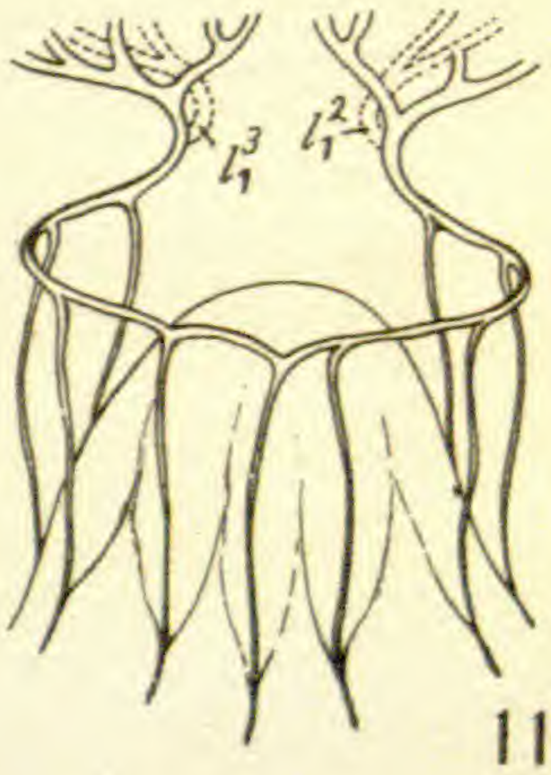

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LANGDON on DIOON

FIG. 5.—Mature wood: radial longitudinal section near lateral border of leaf gap or foliar ray; *p*, pitted tracheids of secondary wood diverted to one side into direction parallel to that of trace; *sc*, scalariform tracheids of trace extending down between fibrous elements of main stele; ×55.

PLATE XVI

FIG. 6.—Mature wood: tangential section of foliar ray; *lt*, leaf trace, scalariform tracheids of trace seen in both longitudinal and transverse section; *x*, primary wood; *ph*, disorganized phloem; *mc*, mucilage duct; ×56.

FIG. 7.—Adult wood: tangential view of foliar ray in vicinity of cambium, tracheids constituting trace seen only in transverse section; ×56.

PLATE XVII

FIG. 8.—Two-year-old seedling, diagram showing: *sl*, origin and course of vascular supply of one of first scale leaves; *L*, first year foliage leaf, and l^2 second year foliage leaf, as traced from cleared material; a, a^1, b, b^1, c, c^1, etc., traces of scale leaf; *cs*, cotyledonary strands; *R*, vascular system of root; ×4.

FIG. 9.—Three-year-old seedling: diagram showing connection of lateral traces, a, a^1, b, b^1, c, c^1, d, d^1, and e, with stem cylinder, and manner of anastomosis to form horizontal girdles; *A*, *D*, two of the four main cotyledonary bundles; *S*, *B*, two of the four principal groups of stem bundles; *lg*, leaf gaps corresponding to foliar gaps of adult stele illustrated in figs. 6, 7; ×4.5.

FIG. 9*a*.—Three-year-old seedling; ×0.5.

FIG. 10.—Median longitudinal section through apical portion of ten-year-old plant, showing only one side of the three sets of horizontal girdles with their associated lateral vertical strands; L_1, L_2, L_3, first, second, and third leaves of crown; second leaf not shown and only part of third leaf; l_1^1, l_1^4, horizontal strands entering adaxial part of leaf one; l_1^2, l_1^3, ventral strands apparently united with the two dorsal girdling strands l_1^1 and l_1^4; ×2.5.

FIG. 11.—Diagram of entire vascular supply of first leaf, L_1, fig. 10; complete anastomosis of leaf traces to form one main horizontal girdling strand; ×2.5.

AUXOGRAPHIC MEASUREMENT OF SWELLING OF BIOCOLLOIDS AND OF PLANTS

D. T. MacDougal

(WITH TWO FIGURES)

The chief purpose of this article is to describe the methods which have been used in the study of colloidal preparations, the reactions of which might furnish a physical basis for the interpretation of growth in plants, and to recapitulate some of the features of swelling of these substances as yet undescribed or but little known.

The investigation of growth in plants involves a measurement of the unsatisfied hydration capacity of living cell masses, and also determinations of the total swelling capacity of desiccated material. In both cases the minute masses of colloids constituting the protoplasts are inclosed in thin walls with a low stretching coefficient. Furthermore, the living cells are in a condition of varying turgidity, dependent upon the osmotic activity of the vacuolar solutions, upon the permeability of the external layer of the protoplasm, and also upon the structure of the walls. Desiccated cell masses have lost the capacity of turgidity as ordinarily known, but may still show some osmotic activity by the differential action of the dead wall (as may other colloids), while the protoplasts have undergone changes due to the action of salts and acids in the concentration of cell saps which accompanies desiccation.

It is not possible to reproduce the mechanical structure of cell masses by artificially compounded biocolloids, so the experimenter must be content to ascertain the general composition of the protoplasm, bring the main constituents together in the form of a jelly, dry this to thin plates, and measure the action of sections of it in solutions of a kind and concentration which would give effects similar to those encountered in living matter.

Physiologists concerned with life in animals dealing with a protoplasm consisting chiefly of proteins and lipins with a characteristic metabolism, have found in gelatine and in the soaps material

which furnishes many valuable homologies. These are in imminent danger of being overworked, however. Not only has gelatine been taken to simulate protoplasm in general, but physicists have committed a similar error of taking the behavior of gelatine as universal for colloids.

Not much progress had been made in the attempt to determine the physical basis of growth in plants before it became plainly obvious that the relations of plant protoplasm to H ion concentration, acidity, temperature, and other conditions diverged widely or were directly the reverse of the action of gelatine.

In seeking other material in a colloidal state which might by its hydration, swelling coefficient, etc., simulate the plant, recourse was had to actual analyses of plants as made in connection with studies in desiccation, starvation, etiolation, and as part of the work on the carbohydrate metabolism of plants by SPOEHR.[1]

The possible importance of the pentoses as a factor in the mechanism of hydration and growth was evident at once. A knowledge of the manner of formation and occurrence of these sugars and of their condensation products, the pentosans (mucilages, slimes, gums, etc.), is necessary in order to understand the rôle they play in the cell.

The metabolism of the plant is predominantly carbohydrate, and the protoplasm may be taken to contain solutions of sugars at all times. Included among the numerous possible changes, it is known that in the depletion of the water content of a plasmatic mass by a general loss from the cell, or by lowered hydration capacity of the colloid by the action of any agent, polysaccharides which have but little imbibition or hydration capacity are reduced to pentosans or mucilages which have a relatively enormous capacity for taking up water. Dextrose, starch, wall material, etc., may be involved in these conversions, and, when masses of material are affected, layers or globules of mucilage may be formed which may react to microchemical tests. It is noted, however, that visible masses of mucilage are of less importance in the mechanism

[1] SPOEHR, H. A., Carbohydrate economy of the cacti. Publ. no. 287. Carnegie Inst. Wash., p. 44.

———, The pentose sugars in plant metabolism. Plant World **20**:365. 1918.

of the cell. The pentosans play their most important part when they are in the form of minute particles in the colloidal mesh of living matter, in which they are still subject to positive but slow changes in metabolism. According to MACDOUGAL, RICHARDS, and SPOEHR,[2] the conversion of other sugars into the pentosans, greatly increasing the hydration capacity of the protoplasm, is the basis of the origination of the xerophytic and succulent types of vegetation.

These facts warrant a procedure in the study of swelling and growth in which various pentosans and pentosan-protein mixtures were subjected to the action of solutions, applied in a concentration and with variations and alternations parallel to occurrences in the cell.

The method of measuring the water capacity of colloidal material includes the following features:

1. Suitable solutions or suspensions are poured at temperatures of 30 to 40° C. into shallow molds to form a jelly.

2. The plates thus cast are dried in a small chamber with a high relative humidity, to a thickness of 0.1 to 0.25 mm.

3. Trios of sections 3×5 mm. in area are placed in dishes of a capacity of 30 cc., a triangular piece of thin glass is placed over the sections, and the vertical swinging arm of an auxograph is seated in the center of the glass. Any change in thickness of the sections moves the levers and moves a pen on a recording sheet carried by the drum.

4. Solutions, the effect of which on the hydration capacity of the sections are to be tested, are poured into the dishes. In nearly all of the experiments in the special range of biological relations it is advisable to renew or replace the solutions at certain intervals.

5. Temperature relations are of the greatest importance, and the record is taken by mercurial thermometers, the bulbs of which are in dishes of liquid similar to those of the experiment.

[2] MACDOUGAL, D. T., and SPOEHR, H. A., The origination of xerophytism. Plant World **21**:245. 1919.

MACDOUGAL, D. T., RICHARDS, H. M., and SPOEHR, H. A., The basis of succulence in plants. BOT. GAZ. **67**:405. 1919.

6. Every measurement obtained by this method is an average of the action of three sections, and has the value of an average. Any value showing notable departures from expectancy should be repeated.

7. Measurements of the swelling of sections by the auxograph give variations in thickness, and serve as direct indices of total changes in volume in plates of agar and agar mixtures, as this material has a strict tendency to return to its original form. Sections of gelatine swell in all dimensions no matter how the plates are cast.

It was desirable to use the best known and most available pentosans or hemicelluloses, and agar-agar, acacia gum, tragacanth, mesquite gum, cherry gum, and mucilage of *Opuntia* were selected for the tests. According to information furnished by H. NAKANO of the Botanical Garden of Tokyo, agar is prepared chiefly from the algae *Gelidium amansii* Lamour, *G. pacificum* Okam, *G. linoides* Kütz., and *Pterocladia capillacea* Born. et Thur., while some material of *Gelidium subcostatum* Kütz., *Ceramium Boydenii* Gepp., *Campylaephora hypenaloides* Y. Ag., and *Acanthopeltis japonica* Okam, etc., may be included. The process includes washing in fresh water, decoloration in the sun, milling, boiling, filtering, maceration in sulphuric or acetic acid, freezing, and drying. Modernized methods simplify this treatment somewhat. Salts, amino-acids, etc., may be present in the final product, and, as it was desirable to reduce the amount of all such subtances, the interest of E. R. Squibb and Sons was obtained, and a purified product was made by the following procedure.[3]

A good grade of commercial agar was dissolved in distilled water and jacketed with superheated steam. The viscous solution produced thereby was filtered clear through a thick mass of steam-jacketed paper pulp, under diminished pressure. The clear solution of agar was dialyzed for about 10 days in a steam-jacketed bath containing running distilled water. After removal of diffusible carbohydrates and salts by the dialysis through parchment paper

[3] For further information concerning the origin and preparation of similar products see SWARTZ, M. D., Nutrition investigations on the lichens, algae, and related substances. Trans. Conn. Acad. Arts and Sci. **16**:247–382. 1911.

of the quality usually employed in the serum industry, the clear solution was slowly poured in a fine stream or spray into 10 times its volume of neutral acetone. The agar was precipitated in the form of fine shreds. It was subsequently extracted with hot absolute acetone, absolute alcohol, and absolute ether. The final product was ground to a granular powder in a porcelain ball-mill with porcelain balls. The resulting preparation contains only a trace of nitrogen and a trace of ash.[4]

Samples of this agar made up as a 2.5 per cent solution with distilled water had a light brown tinge, dissolving completely within an hour at about 100° C. It was found that the material made up as a 0.75 per cent solution, when poured into a test-tube, formed a "slant" which kept in place when the tube was set in an upright position at 15° C. for 2 weeks. Layers 1 cm. deep in small flasks retained their form when the flasks were inverted in some instances. Some water was separated, gathering on the upper part of the tube or flask, and the appearance of same on the surface of the agar suggested syneresis. Tests of this agar by the colorimetric process of DUGGAR gave it a hydrogen ion concentration denoted by $P_H = 6.5$.

The mucilage of *Opuntia*, cherry gum, mesquite gum, *Acacia*, and tragacanth gum was extracted or dissolved in water (nearly always showing a solid residue), precipitated with alcohol, filtered, and dried in a desiccator. These substances go into solution at lower temperatures than agar, and differ from it in many important features, as may be seen in the table of swelling reactions.

Special preparations were also made of salt-free water-soluble albumins from oats, wheat, the common bean, soy bean, and castor bean, as well as of lipins and amino compounds, by methods commonly used and need not be described here. The gelatine used was the "bacto-gelatine" now available in quantity.

The measurement of the swelling of these colloids must take into account the structural features resulting from dehydration. Sections or plates of agar, for example, tend to return to the form and dimensions of the layer of watery gel from which they were produced. This is not true of gelatine, as will be described later,

[4] The description of this process was furnished by Dr. ISAAC HARRIS.

nor can it be said of the other mucilages or gums already mentioned as they go into solution from the surfaces of the sections; but sections in which agar forms as much as half may be assumed to return to the original in nearly all work, with a change of not more than 2–5 per cent in area (fig. 1).

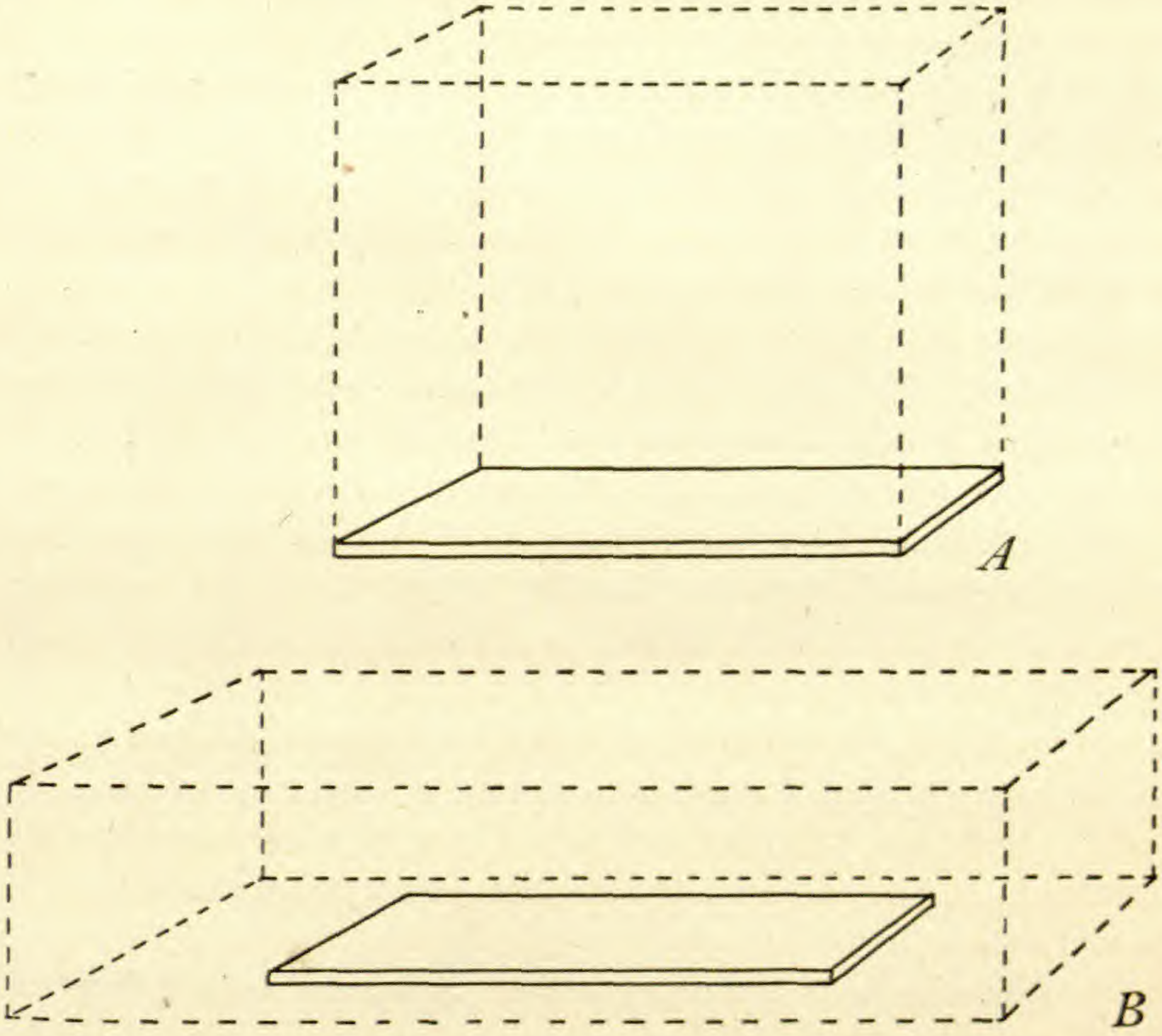

FIG. 1.—Diagrams illustrating action of dried plates of agar and gelatine in swelling: in *A*, section of agar, represented by thin plate forming bottom of figure, swells to larger block by increase almost wholly in thickness; in *B*, gelatine plate represented by small inner figure increases not only in thickness but also in length and breadth to form larger block of jelly.

In view of these facts, it is evidently desirable to cast plates and dry sections in such manner that the dried sheet of material will have the areal dimensions of the original, and that all of its shrinkage will have taken place in such manner as to reduce the thickness only. The preparation of such plates may be done simply by pouring warm solution on a glass plate, where it hardens,

dries, and adheres. If it does adhere in such manner as to prevent areal shrinkage, it generally is to be removed with difficulty and may be destroyed in the process.

An improved method of making such plates is briefly as follows. First a sheet of gold foil is laid out on a leveled glass plate on a table. Blocks of glass or of some non-corrosive metal are placed on the margins of the gold. The warm agar is poured into this cell in a 2.5 per cent solution, at 40° C., and in cooling to 18 or 20° C. it sets, and the blocks may be removed. The gel now stands on a base of gold foil, and unless anchored at the margins will shrink in all dimensions as it dries, a thing which specifically is to be prevented. To do this a little warm solution of agar is run around the agar plates which on cooling cements the margin to the glass. The preparation is now set in a dehydrating chamber with a humid atmosphere and subjected to the action of an electric fan for 40 hours. At the end of this time it has come down to a plate about one-fortieth of its original thickness, and may be detached from the plate by cutting away the marginal portion. Properly made, such plates are even as to thickness and swelling qualities.

When albumin, gelatine, or other albuminous substances are to be mixed with the agar, the solution of the latter is cooled to 40° C., and then the other substances in a liquefied condition are poured in and stirred for a few minutes before the plate is poured, which should be done at about 35° C.

The process puts the experimenter in possession of a sheet of dried material, preferably between 0.15 and 0.20 mm. in thickness, and after the margins are trimmed away with the scissors the sheet will probably have a surface of about 7×12 cm. As indicated, these sections may be about 3×5×0.16 mm., with a volume of 2.4 cu. mm., and the trio in a dish have a total of 7+cu. mm., into which a measured amount of solution is poured and replaced as the experiment demands. If thinner sections are used, their area should be reduced.

The auxograph consists essentially of a compound lever set with a vertical swinging arm which rests on the triangular glass plate covering the sections in the dish, and its use in measuring

the swellings is a means of recording phenomena not to be evaluated in any other manner[5] (fig. 2).

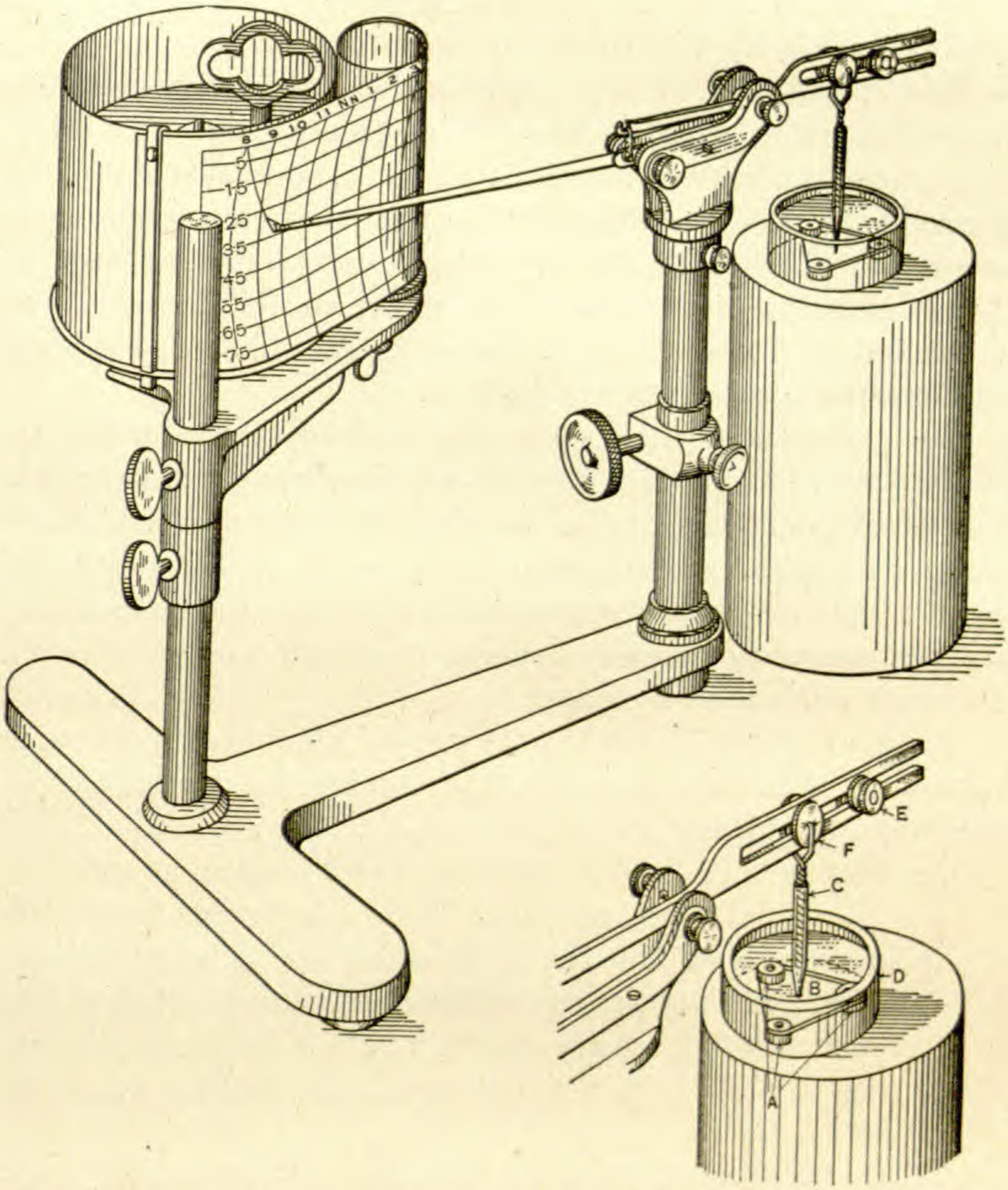

FIG. 2.—Auxograph arranged to record changes in thickness of trio of sections of colloidal material; tip of pointed glass tube, sealed to vertical swinging arm *C* (smaller figure), rests in socket in center of thin triangular glass plate *B*, which lies on sections *A;* expansion of sections pushes this arm upward (adjustment at *F* placed to give an amplification of 20 in cut), compound lever being arranged to give a downward movement of pen; record slip, daily or weekly, 8 cm. wide, is ruled to mm. (not shown in cut). Illustration made to show action of trio of sections of agar in water at end of 1.5 hours.

[5] For description of this apparatus see MACDOUGAL, D. T., Hydration and growth. Publ. no. 297. Carnegie Inst. Wash. 1919.

Thus, for example, instead of the common method of taking the total gross volume of a colloid in a dish or test-tube, the auxograph calibrates the action of sections like those of agar, which swell almost wholly in one axis (and a related heterotropic swelling is highly probable in all protoplastic action), and records the continuous rate of swelling. This gives the experimenter exact information on many features, of which the practical cessation of swelling is one of the most important. Thus some of the records show a swelling at a decreasing but notable rate for as long as 20 days. The termination of the experiment at the end of the second or even the tenth day would have eliminated some of the most striking and important features of the reaction.

Such continuous records are also necessary in following the action of solutions in which the mass relations are such as to require renewal of the liquids, and in the study of plates in which amino compounds, salts, etc., have been incorporated. Of other features, by no means the least advantageous is the use of the same instrument in measuring changes in volume by growth swelling of living and dried cell masses of plants.

A set of results of swelling of various biocolloids is given in table I, in which the increases, first obtained in percentages of the original thickness of the dried material, are converted into figures relative to the swelling in water which is taken as 100.

The P_H values were calculated from colorimetric tests after the indicator method perfected by DUGGAR,[6] soy albumin showing a value of 6.2 in a 0.5 per cent solution, *Opuntia* mucilage 5.8 in a 1 per cent solution, cherry gum 5.1 in a 1 per cent solution, acacia gum 5.1 in a 1 per cent solution, and gelatine 5.2 in an 8 per cent solution.

These biocolloids are to be regarded as intimately mixed particles, strands, webs, or globules of pentosans and of proteins, as these substances do not unite and are not mutually interdiffusible. The combinations of material were so arranged as to

[6] DUGGAR, B. M., and DODGE, C. W., The use of the colorimeter in the indicator method of H ion determination with biological fluids. Ann. Mo. Bot. Gard. 6:61–70. 1919.

DUGGAR, B. M., Refinements in the indicator method of hydrogen ion determination, Rept. Dept. Bot. Research, Carnegie Inst. Wash. 1919.

give illustration of the special character or dissimilarity of constitution of these mucilages when used to replace part of the agar. First it is to be seen that in the biocolloids in which acacia, cherry, and mesquite gum replace one another, the reactions to high H ion concentration in the acid and to potassium hydroxide are not widely different in total amount. The coefficient of increase in

TABLE I

RELATIVE SWELLING OF BIOCOLLOIDS IN ACIDS, HYDROXIDES, SALTS, AND WATER AT 15° IN 0.01 N CONCENTRATION; INCREASE IN WATER GIVEN IN PERCENTAGES OF ORIGINAL DRIED THICKNESS OR VOLUME

Material	Parts	KOH	NH_4OH	KNO_3	HNO_3	HCl	Water
	P_H	11.99	10.61	6.6	2.01	2.01	
Agar	10	33	83	39	44	44	(1800)
Gelatine	10	200	197	80	245	300	(1570)
Agar	8	50	45	45	32	23	(2000)
Soy bean albumin	2						
Agar	4	19	25	47	22	22	(2785)
Opuntia mucilage	4						
Soy bean albumin	2						
Agar	4	29	41	58	26	28	(2415)
Cherry gum	4						
Soy bean albumin	2						
Agar	4	33	36	67	31	29	(2100)
Acacia	4						
Soy bean albumin	2						
Agar	4	110	91	96	48	48	(1100)
Acacia	4						
Gelatine	2						
Agar	3	75	90	67	50	56	(1200)
Acacia	3						
Soy bean albumin	2						
Gelatine	2						

potassium nitrate does vary widely, however. The special effects in ammonium hydroxide are discussed elsewhere.[7]

When these mixtures are viewed as homologues of cell masses or of plant protoplasm, that containing the *Opuntia* mucilage

[7] MACDOUGAL, D. T., and SPOEHR, H. A., The effect of organic acids and their amino compounds on the hydration of agar and on a biocolloid. Proc. Soc. Exper. Biol. and Med. **16**:33. 1919.

assumes a special interest, as the total swelling in water is greatest of all of the combinations, and the depressing effects of acid, hydroxide, and salt are very marked. These features would be characteristic of a plant capable of a wide range of water content and sensitive to changes in the sap, as is known to be the case from studies of the growth of *Opuntia*.

The substitution of gelatine for albumin, or its addition to a mixture increases swelling in acid, salt, and hydroxide as would be expected, and lessens the total swelling in water. These and other available data may be profitably construed in many directions in the interpretation of growth phenomena.

The methods of preparation and measurement of swelling of colloids described have served to confirm and extend knowledge of the behavior of agar, albumin, gelatine, and of mucilages, and to fix upon pentosan-protein mixtures which swell in a manner similar to cell masses of plants. The use of the auxograph has made it possible to compare the nature, extent, and duration of these changes with variations in volume of growing cell masses.

The casting and desiccation of colloidal plates in such manner that shrinkage and swelling takes place unequally in different axes, and the measurement of such differential swelling also furnishes some evidence which may be of value in interpreting the changes in form, etc., of the special bodies of the protoplast which accompany and mark the morphological crises of the cell.

DESERT LABORATORY
TUCSON, ARIZ.

HAWAII'S TAPESTRY FORESTS

VAUGHAN MACCAUGHEY

(WITH SIX FIGURES)

Many of the Hawaiian mountains are deeply eroded. Torrential rainfalls, operating throughout vast periods of time, have strongly carved the original volcanic domes. Extraordinary precipices (called *pali* by the natives) abound in all parts of the islands. Fantastically sculptured canyons, ravines, and gorges, profusely cliffed and ramified, are characteristic of the montane areas. The valley walls are notably abrupt. Many of the valley heads are rimmed with cliffs and crags. The summit ridge on such islands as Kauai, Oahu, and Molokai is worn in many places to a thin crest, with numerous lateral "razorback" ridges.

Associated with this highly dissected topography is the subtropical montane rain forest. The general features of this forest have been presented by the writer in other papers.[1] Herein are described only those portions of the rain forest that cling to very steep slopes.

The naturalist, exploring the Hawaiian forests, is sure to be impressed by the ability of the groves to maintain themselves on very precipitous slopes. Although the individual trees are of small stature, with abundant shrubby undergrowth, all of the area is closely occupied, forming an unbroken arborescent or semi-arborescent mantle. The writer proposes the name "tapestry forest" for this particular forest type, that successfully occupies almost vertical mountain walls. All tapestry forest is montane rain forest, but all rain forest is not tapestry forest. Indeed, the most luxuriant rain forest is on gently sloping uplands (for example, Puna and Olaa, Hawaii); the trees of this *Metrosideros-Cibotium* formation attain heights of 75–90 ft. The tapestry groves of course do not grow on absolutely vertical cliffs. Seen from a

[1] Survey of the Hawaiian land flora. BOT. GAZ. **64**:98–114. 1917; Oahu rain forest. Amer. Forestry **23**:276–278. 1917; Economic woods of Hawaii. Forestry Quarterly **14**:696–716. 1916.

distance, the slopes mantled with vegetation appear to be much steeper than they actually are. The actual slopes range between 40 and 80°, averaging 50–60°. The vertical walls are either totally bare, or, if somewhat roughened and ledgy, support a depauperate, windswept, scattering growth of hardy grasses, ferns, and bryophytes. The moist areas, produced by seepage waters, are habitats of algae, lichens, and mosses.

The finest examples of tapestry forest occur in the following situations. On the island of Kauai: the Na Pali district, Wainiha,

FIG. 1.—Tapestry forest on deeply eroded mountain ridges

Hanalei, and on the upper summit ridges and cliffs of Waialeale. On the island of Oahu: the windward precipices of the Koolau Range, and such valleys as Makaha, Makua, and Waianae, in the Waianae Range. On the island of Molokai: the precipices and summit slopes of such valleys as Halawa, Pelekunu, Wailau, and Kalawao. On the island of Maui: the valleys of Iao, Waikapu, Waihee, and the summit slopes of West Maui; also the windward and eastern valley slopes of East Maui. On the island of Hawaii: Waipio and Waimanu valleys, and other precipitous slopes along the Hamakua coast.

In altitudinal range the tapestry forests lie mostly between 800 and 4500 ft., in hygrophytic situations. In certain localities, as along the Hamakua coast, they extend to sea-level. In numerous situations along the Hawaiian coast are cliffs and low peaks, now arid and xerophytic, which give evidence of having been covered by tapestry rain forest in prehistoric times.

The steepness, wetness, and general inaccessibility of the tapestry groves have prevented wild cattle and goats from ravaging them. Thus they have been spared the devastations so

FIG. 2.—Oahu tapestry forest; montane rain forest

abundant and irreparable in the lower forests, and retain a much more primitive aspect. The undergrowth, although relatively scanty, and composed of smaller individuals than is the undergrowth of the lower forest, is particularly interesting because of its primitive and undisturbed character.

Closely related to the tapestry groves, both in ecological characters and in floral content, are the groves which inhabit the steep-walled hanging valleys, or high ravines, that are such a characteristic feature of the Hawaiian montane topography. These steep glens, lying at elevations of 800–3000 ft., do not terminate on the level of the valley floor into which their waters debouch, but on the face of

sheer cliffs, 100–2000 ft. high. The streams fall over these cliffs in beautiful cascades. The rain forest in the hanging valleys is not subjected to ecological conditions as severe as those of the tapestry forest, and hence attains more normal development.

The tapestry groves are notably dwarfed, with the aspect of marked and premature senility. The conditions of the substratum

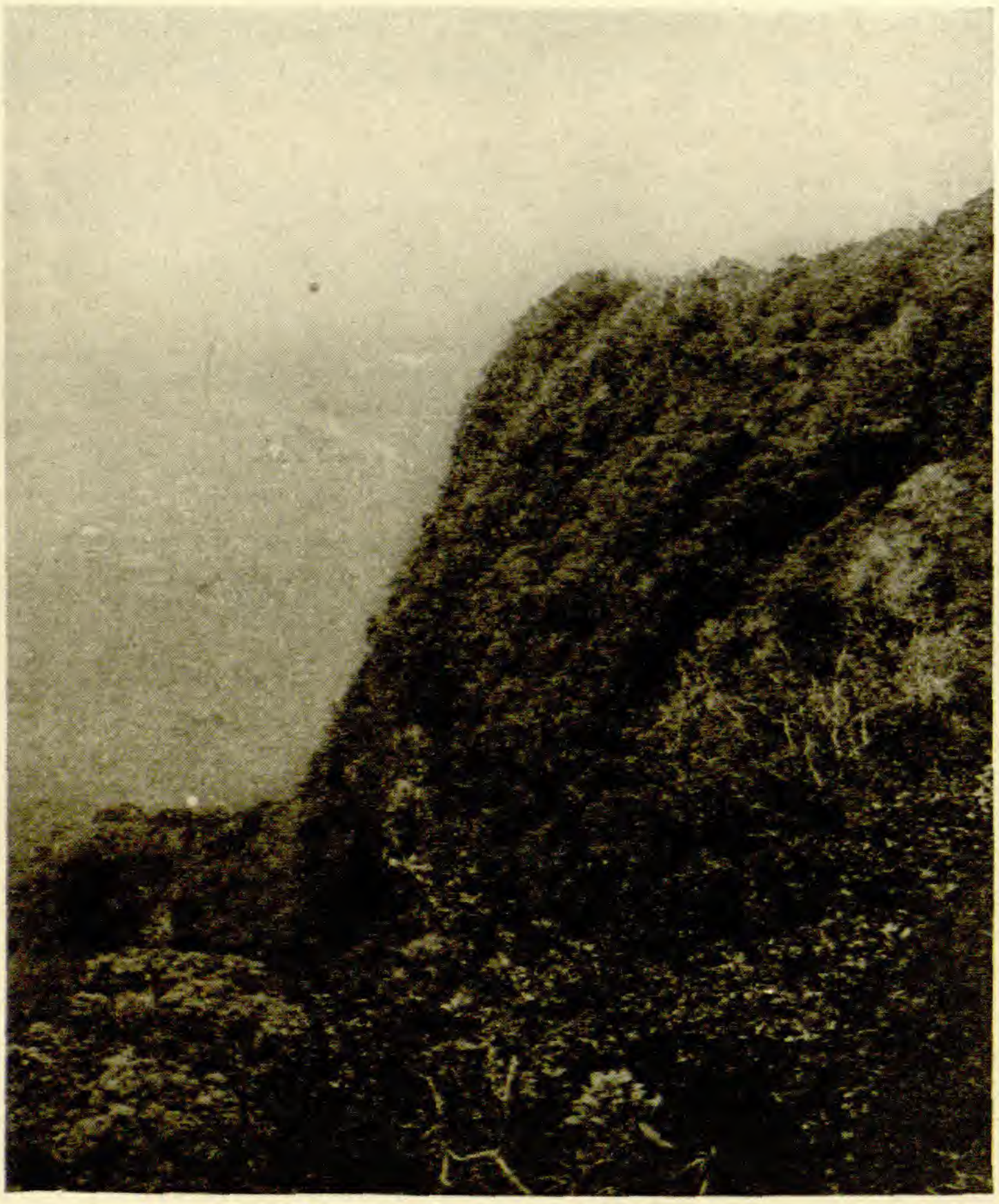

FIG. 3.—Tapestry groves on very steep lateral spurs

afford but precarious roothold, and are obviously unfavorable for normal arborescent development. Many species that in sheltered level regions reach heights of 50–80 ft., with large trunks and symmetrical crowns, are stunted, gnarled, and dwarfed to a marked degree on the precipices. The trunk is short, usually leaning outward from the cliff, rarely erect, and breaks up into a number of

wind-shaped branches. Most of the steep slopes are subjected to strong winds, which impress their mark upon all aerial parts of the vegetation. Very young trees and old dead trees are alike rare in the tapestry groves, the area being so closely occupied by mature trees. Conditions are unfavorable for seedlings, and reproduction is conspicuously retarded. Weakened or dead trees soon lose their roothold, and fall from the grove into the lowlands below. The appearance of senility is in part fictitious, as none of the trees give

FIG. 4.—Tapestry groves on valley wall, Kalihi, Oahu

evidence of being more than 100–150 years old, and the younger trees (10–50 years) soon acquire an aged and decrepit appearance in their ceaseless struggle against gravity and wind.

The observer, standing on the floor of a valley and looking at the forest mantle which drapes the slopes, is impressed by the various shades of green in the mottled canopy. All of the tree crowns are domed or hemispherical; there are no conifers or cycads. Most of the crowns are more or less highly ramified. The foliage in the majority of cases is composed of small, simple, oval, glossy

leaves. Thus the summits or crowns of the various species look very much alike, save for the differences in the green tints. These

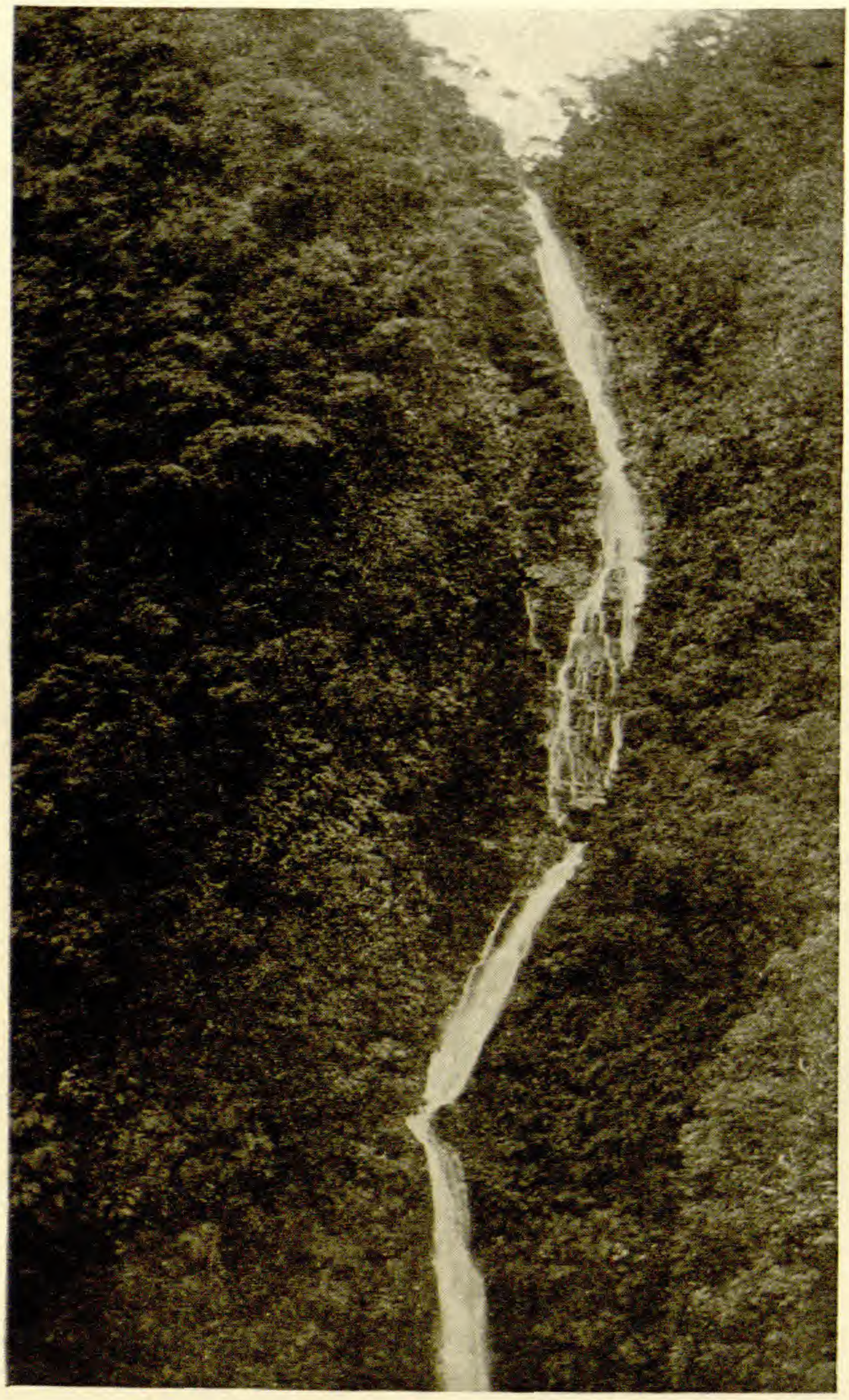

FIG. 5.—Vertical wall, with cascade and tapestry formation

differences are much more pronounced than in temperate zone forests, and give to the tapestry a distinctive and singular charm. Gray-green, yellow-green, olive-green, silver-green, green flushed

with red, heavy somber green, glossy green, literally scores of subtle and indefinable shades of green are discernible to the prac-

FIG. 6.—Arid ridges and precipices, formerly forested, devastated by goats and cattle

ticed eye. These varied hues give to the groves, particularly in the slant light of late afternoon, a rich, mottled, velvety effect, as though the forest were indeed a wondrous woven drapery.

A vertical or cross-section through a typical grove would reveal the following strata. (1) The basaltic rock cliff or wall, made up solely of ancient lava beds, piled one upon another. (2) A thin layer of lava soil, 4–12 inches thick, yellowish or reddish stiff clay, and excessively retentive of moisture. This soil is derived directly from the underlying lava. When stripped of vegetation, as by landslides, it washes away very rapidly, and exposes the rock strata. (3) A very thin layer of vegetable mold, not exceeding 2–6 inches. Conditions in the rain forests are not favorable for the accumulation of humus, and on the steep slopes occupied by the tapestry groves, very little accumulation is physically possible. (4) The vegetable mold or forest floor (in reality more of a wall than a floor) is covered with various hygrophytic species of lichens, liverworts, mosses, filmy ferns, coarser ferns, and a few herbaceous-perennial seed plants. Among the latter are such genera as *Astelia*, *Gunnera*, *Liparis*, *Peperomia*, *Nertera*, etc. (5) The forest proper, consisting of small trees, shrubby trees, shrubs, and lianas. The following are representative members of the Hawaiian tapestry grove formations:

Acacia koa Gray
Aleurites moluccana Willd.
Alyxia olivaeformis Gray
Antidesma platyphyllum Mann
Bobea elatior Gray
Broussaissia arguta Gaud.
Charpentiera ovata Gaud.
Cheirodendron Gaudichaudii Seem.
Cheirodendron platyphyllum Seem.
Claoxylon sandwicense Mueller
Clermontia spp.
Coprosma spp.
Coreopsis spp.
Delissea spp.
Dodonaea viscosa L.
Dubautia laxa Hook. and Arn.
Dubautia plantaginea Gaud.
Elaeocarpus bifidus Hook. and Arn.
Eugenia sandwicensis Gray
Euphorbia Hookeri Steud.
Euphorbia multiformis Hook. and Arn.
Euphorbia spp.
Eurya sandwicensis Gray
Exocarpus brachystachys Hbd.
Freycinetia Arnotti Gaud.
Gardenia Brighamii Mann
Gardenia Remyi Mann
Gouldia spp.
Hesperomannia arborescens Gray
Hibiscus Arnottianus Gray
Hibiscus kokio Hbd.
Ilex sandwicensis Loes.
Kadua spp.
Labordia spp.
Lipochaeta connata DC.
Lipochaeta decurrens Hbd.
Lipochaeta spp. and vars.
Lobelia spp.
Lysimachia Hillebrandia Hook. f.
Lysimachia spp.
Maba sandwicensis A.DC.
Metrosideros macropus Hook. and Arn.
Metrosideros polymorpha Gaud.

Metrosideros rugosa Gray
Metrosideros tremuloides Rock
Nothocestrum spp.
Ochrosia sandwicensis Gray
Osmanthus sandwicensis Knobl.
Osteomeles anthyllidifolia Lindl.
Pelea spp.
Perrottetia sandwicensis Gray
Phyllostegia spp.
Pipturus albidus Gray
Pisonia umbellifera Seem.
Pittosporum spp.
Plectoronia odorata Benth. and Hook.
Platydesma campanulata Mann
Platydesma cornuta Hbd.
Pritchardia spp.
Rauwolfia sandwicensis A.DC.
Reynoldia sandwicensis Gray
Rollandia spp.
Santalum Freycinetianum Gaud.
Santalum spp.
Scaevola spp.
Scheidea spp.
Sideroxylon spp.
Smilax sandwicensis Kunth.
Solanum sandwicense Hook. and Arn.
Stenogyne spp.
Straussia spp.
Styphelia tameiameia F. Muell.
Suttonia spp.
Tetramolopium spp.
Tetraplasandra spp.
Urera sandwicensis Wedd.
Vaccinium penduliflorum Gaud.
Viola spp.
Viscum articulatum Burm. and vars.
Wikstroemia spp.
Xanthoxylum spp.

Among the ferns of the humid forests, the following are likely to occur in the tapestry groves:

Adiantum capillus-veneris L.
Asplenium spp.
Athyrium spp.
Botrychium subbifoliatum Brack.
Ceropteris spp.
Cibotium Chamissoi Kaulf.
Cibotium Menziesii Hook.
Cibotium glaucum Hook. and Arn.
Coniogramme spp.
Cyrtomium Boydiae Robins.
Dicranopteris spp.
Diellia pumila Brack.
Diellia falcata Brack.
Doodia Kunthiana Gaud.
Doryopteris spp.
Dryopteris spp.
Elaphoglossum spp.
Filix Douglassii Robins.
Hymenophyllum spp.
Hypolepis punctata Mett.
Lycopodium spp.
Marattia Douglassii Baker
Microlepis spp.
Neottopteris Nidus J. Sm.
Odontoloma Macraeanum Brack.
Ophioglossum pendulum L.
Pellaea ternifolia Link.
Phymatodes spp.
Polypodium spp.
Psilotum complanatum Sw.
Psilotum nudum Griseb.
Ptedium aquilinum Kuhn
Pteris cretica L.
Sadleria cyatheoides Kaulf.
Sadleria Hillebrandii Robins.
Sadleria polystichoides Heller
Sadleria spp.
Schizaea robusta Baker
Selaginella spp.
Tectaria cicutaria Robins.
Trichomanes spp.
Vittaria rigida Kaulf.

Of particular interest, from the ecological viewpoint, are the root systems of the tapestry grove trees and shrubs. The combination of steep declivity, thin clay soil, and rock substratum necessitates the development of an unusually strong mechanical root-supporting system. Most of the trees have a number (5–12) of large proplike roots which extend downward below the trees, and are firmly rooted in interstices in the ancient lava beds. These lower roots brace the tree staunchly from below. On the upper side of the trunk are usually several long anchoring roots, more or less exposed in the thin vegetable mold. The extremities of these roots are likewise rooted among the rock strata. In some situations, where the processes of erosion are gaining upon the grove, and have washed away much of the grove floor, the intricate systems of anchoring and bracing roots are beautifully displayed. On many cliffs and steep ridges these strong roots form a sort of natural ladder, well known to the natives and woodsmen, who utilize them in ascending or descending the slopes. In many situations ascent would be well-nigh impossible were it not for these tough, firmly anchored, exposed roots.

Lianas of various species establish themselves in the tapestry groves, and in some places become so luxuriant as to form almost impenetrable hanging jungles. Conspicuous among these vines are species of *Freycinetia*, *Dicranopteris*, *Smilax*, *Dioscorea*, *Alyxia*, *Ipomoea*, *Convolvulus*, etc. The liana formations are best developed at the lower levels; above 2000 ft. the groves are practically free from vines, which are replaced by the dense soggy moss formations.

The tapestry groves, owing to the hygrophytic environment, are usually heavily clad with thick layers of epiphytic lichens, mosses, filmy ferns, and liverworts. These layers, on the smaller branches and saplings, are often 4–8 inches in radius. The outer layer alone is green and living; the under layers, of dead vegetable material, are saturated with rain water, and may be wrung out like a wet sponge. This moss covering is best developed at the higher altitudes (1800–4000 ft.), where the annual precipitation is 100–400 inches. These upper tapestry groves are swathed in fog and rain during most of the year, and comprise a range of woody species different from that of the lower groves.

Landslides are perpetual enemies of the hanging groves. They cut short the lives of the trees which they undermine, and expose fresh rock surfaces to soil-making and revegetation. The landslides vary in width from 6 to 60 ft., and in length from 25 to 1000 ft. Owing to the slow rate of reproduction in the rain forest species, and the inhospitable climatic and soil conditions for seedlings, these rents in the tapestry are slow healing. Commonly various grasses and such ferns as *Dicranopteris* and *Sadleria* are the first invaders, followed by herbaceous-perennial seed plants (*Dianella*, *Coreopsis*) and shrubs. The arborescent species come last, and very slowly.

The two great activities of subsidence and erosion are gradually but inexorably reducing the areas occupied by the tapestry groves. The base-leveling action of erosion tends to flatten all slopes. The immeasurably slow subsidence of the entire archipelago has shrunk the original heights of the mountains. Thus, from the historical viewpoint, tapestry groves represent a transient ecological phenomena. At present it is possible to find every stage, from steep walls covered with luxuriant endemic woody tapestry, to flattened earthy hills, clad only with foreign grasses, and ranged by cattle.

The tapestry groves are of large economic value as earth protectors and water conservators. Their scenic beauty alone would amply warrant their stringent protection. As water sheds they are of much local significance. They comprise one of Hawaii's most distinctive and lovely natural assets.

HONOLULU, HAWAII

STATOCYTES OF THE WHEAT HAULM

T. L. PRANKERD

(WITH FOUR FIGURES)

The plant statocyte is a cell containing a body or bodies, the statoliths, free to move within it under the force of gravity (**1, 5**). In the mature wheat haulm, the statocytes are entirely confined to the "nodes"[1] or swellings of the leaf sheaths just above their attachment to the stem. Here they occupy more than half the total bulk, forming a definite and continuous tissue, which I have previously termed statenchyma (**5**).

Fig. 1 makes the anatomy in this region clear. The swollen leaf sheath is bordered within and without by an epidermis succeeded by a few layers of collenchyma of the type described by HABERLANDT as lamellar (**2**, p. 156). Between these layers the fibrovascular strands, consisting mainly of slightly lignified fibers, are arranged in an irregular ring, varied occasionally by smaller strands of similar fibers unaccompanied by, or surrounding a trace only of vascular tissue. The whole, or nearly the whole of the ground tissue is transformed into statenchyma, more highly differentiated than usual, since the statoliths are of two kinds. The more general type of statolith (the starch grain) is found in groups of cells occurring adaxially to the vascular strands. These sometimes form only one or two layers, but occasionally are more extensive, and even reach the internal collenchyma (fig. 1). All the other statocytes, composing by far the greater part of the ground tissue, contain crystal statoliths, and are best developed internally to, and on the flanks of the fibrovascular strands.

This position of the statenchyma within the vascular ring is unusual, although not without parallel.[2] A crystal statolith, however, is probably extremely rare, for, although expecting it, I have

[1] The term "node" is used throughout the paper in this sense.

[2] In 1911 I figured (**3**) the statocyte pith of *Hottonia palustris*, and since then other cases have become known to me of the conversion of the pith in whole or in part to statenchyma.

never discovered it elsewhere, notwithstanding careful search in more than a hundred plants, widely differing in habit and systematic position.

The crystal-containing statocytes are nearly twice as broad as they are long, that is, they are compressed in the axial plane. In shape they are irregularly cylindrical and hence circular in transverse section, while the longitudinal walls are generally more or less gabled (cf. fig. 3). The shape and arrangement of these cells thus allow easy extension of the tissue on bending of the node. The average diameter of the cells is about 55 μ, and the height about 30 μ. The walls are pitted, which is best seen in transverse sections showing the flat horizontal walls in surface view. Each contains a relatively large nucleus (about 18 μ diameter). In one case two nuclei were seen, although to what extent this is a general phenomenon in the wheat plant has not yet been determined (cf. **4,** note at end). The crystals usually occur singly in the cells, and when there are several this is probably due in some cases to fracture. They apparently belong to the tetragonal system (10 μ diameter), and occur as prisms, pyramids, spherical aggregates, combinations of these, or in less definite forms (fig. 4). Hydrochloric acid dissolves them at once, but they are unaffected by glacial acetic acid and various stains. From these facts their chemical composition is judged to be calcium oxalate.

The amylostatocytes decrease in size as they approach the vascular tissue, and are of much smaller average diameter than the crystal statocytes (fig. 2). Each possesses a nucleus (about 10 μ diameter), and numerous starch grains occupying about half the volume of the cell. The grains are simple, spherical, and about 5 μ in diameter. Movable crystals may also occur in these cells.

Search has been made for starch and crystals in other parts of the mature wheat plant. The latter occur in the pith of the stem, but are not free to move; and, except of course in the grain, only traces of imbedded starch have occasionally been found. Preliminary work on the seedling and young plant indicates that statolith starch exists throughout its life and appears in the same locality, that is, it is constant in time and space, while imbedded

starch is neither. The latter naturally tends to disappear from all parts while the grain is filling, and it is therefore a fact to be noted that the statolith starch is unaffected by this drain on the stored food material, and therefore cannot be regarded as primarily nutritive in function.

Both DARWIN and HABERLANDT have referred to the "falling time" (**1**, p. 771), and "period of migration" (**2**, p. 598) of statoliths, but I am not aware of any work on the *rate* of travel other than my own, which is not yet published in full (**6**). The unique feature of the wheat plant in possessing two kinds of statoliths, together with its extreme economic importance, perhaps justify a separate statement at the present time. Experimental work has demonstrated the fact that the rate of travel of the crystal is much greater than that of the starch grain, for the latter falls at about 120 μ per hour, while the rate of the former is nearer 600 μ.

The rate of the starch grain approximates to that obtained for the movable starch grains in the inflorescence axis of *Lupinus*, and is probably very average, but 600 μ per hour is about three times as great as that of any statolith known to me.

The actual time taken for the crystal to travel from one side of the cell to the other is probably under 5 minutes, and the same period for the starch grain varies with the diameter of its statocyte, but averages about 15 minutes. In nature, however, since the statocyte could never be displaced more than 90° from the vertical, the statolith would never traverse the whole diameter of the cell, but some distance less than this, and hence would reach its new position in a period of time less than that stated.

If the impact of the falling body on the living protoplasm lining the cell is a means of perception by the plant of the direction of gravity (**1, 2**), it is the latter period which is of biological importance. It then becomes interesting to note that this period is the shortest on record. The wheat is capable of bending at the nodes in order to bring the haulm into a vertical position should it be displaced; and it is an obvious saving of energy in the transmission of stimulus that these organs should be at the same time both sensory and motor, that is, capable of perceiving and also of acting upon the appropriate stimulus.

The recognition of the node as indeed a definite sense organ of gravity perception on the part of the wheat plant readily accounts for the presence of starch in this region when the ear is ripe, which otherwise seems difficult of explanation.

I would further suggest the possibility that in the course of evolution the wheat plant may be substituting a body, metabolically harmful, but heavier and therefore quicker and better as a

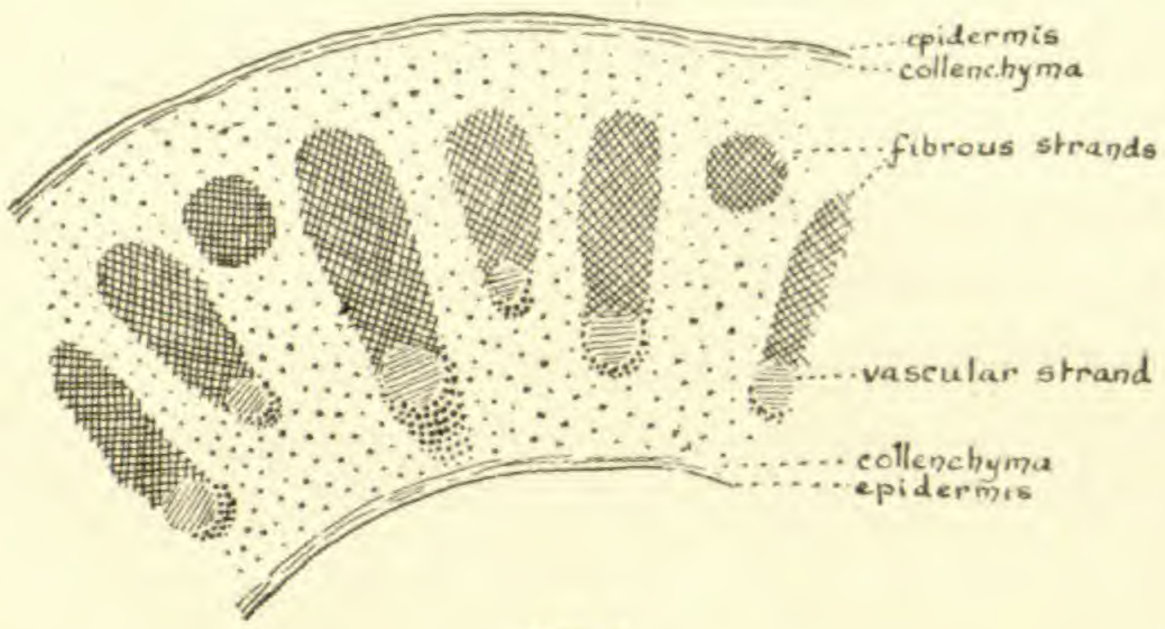

FIG. 1

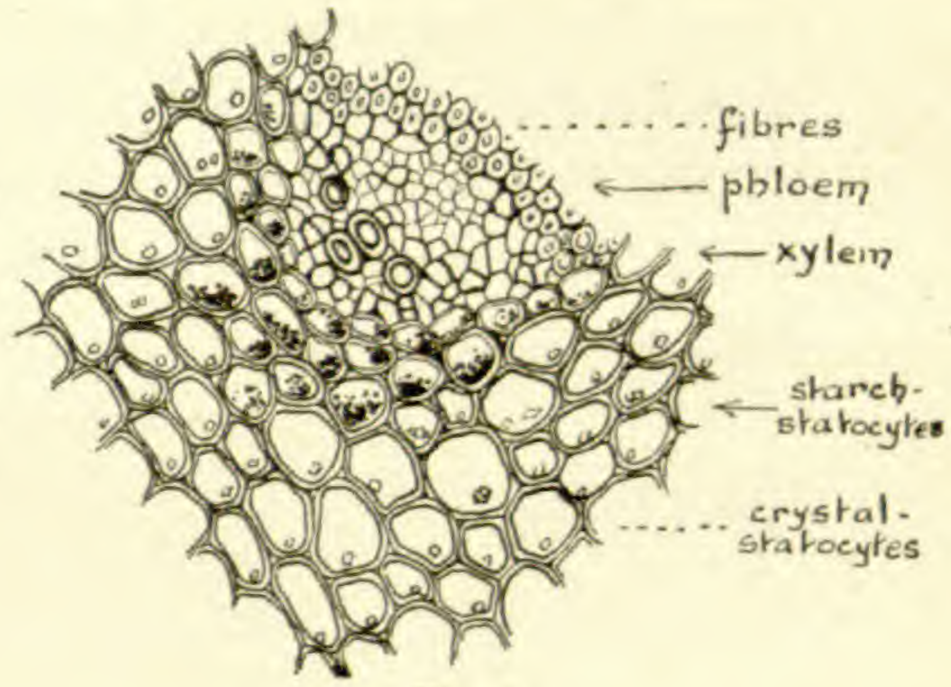

FIG. 2

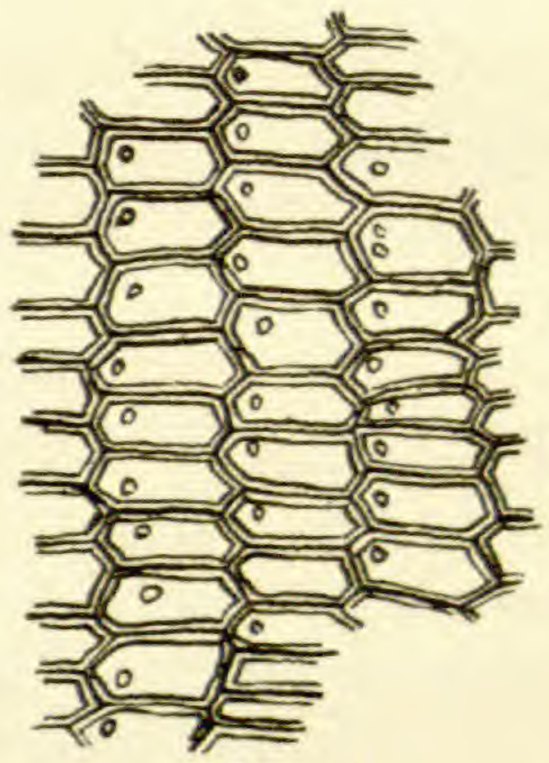

FIG. 3

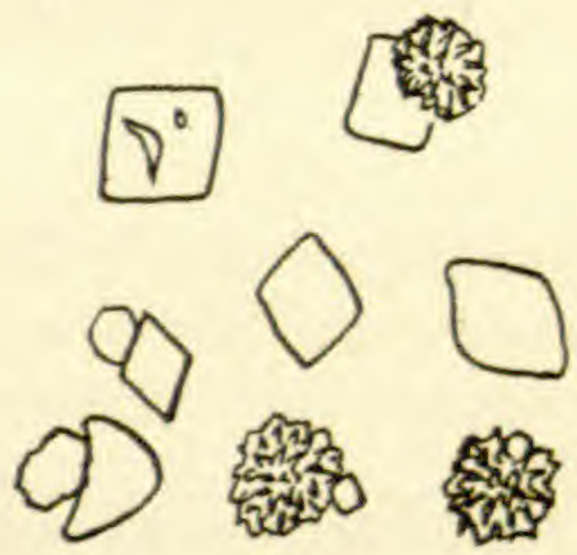

FIG. 4

FIGS. 1–4.—Fig. 1, part of transverse section (diagrammatic) across node of wheat stem, showing position of statenchyma: heavily dotted line, amylostatenchyma; lightly dotted line, crystal statenchyma; ×20; fig. 2, portion of fig. 1 enlarged to show structure; ×70; fig. 3, portion of crystal statenchyma in longitudinal section; ×140; fig. 4, various forms of crystal statoliths; ×620.

statolith, for the usual starch grain, which is required as nutriment for the ear. If this be true for grasses in general, it may be one of the many subtleties of structure that have contributed to the extraordinary success of the group. I am not without hope that future work may not only establish the nodes of the wheat plant

as sense organs, but may even show that they have some bearing, even if remote, on such a practical problem as the lodging of crops.

Summary

1. The wheat haulm possesses two types of statocyte: (1) the smaller containing movable starch grains, (2) the larger with one movable crystal of calcium oxalate. Both occur only in the nodes.

2. The rate of fall of the crystal is much greater, and the period of migration considerably less than the corresponding quantities for the starch grains.

3. It is suggested that the nodes of the wheat are definite sense organs, showing a high degree of evolutionary development, and that future study of the reaction to gravity of the plant should take them into account.

Part of this investigation was carried out in the field and laboratory at Rothamsted Experimental Station, and I gratefully acknowledge the kindness of Dr. E. J. RUSSELL and members of his staff in affording me every facility while working there.

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READING, ENGLAND

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BRIEFER ARTICLES

RELATION OF FLAX TO VARYING AMOUNTS OF LIGHT

Certain features of a plant's metabolism take place in sunlight or diffuse daylight, while other changes go on during the period of darkness. In this connection the question arises, Is the amount of light (measured in duration, but not in intensity) received by a plant at any part of the earth's surface ever so prolonged as to serve as a check to the plant's growth? In other words, if the duration of the periods of darkness and light were approximately equal, would the plant make as much growth as in those cases where the light period is greatly in excess of the darkness period? In other words, does the prolonged exposure of a plant to light in a more northern latitude compensate to a considerable extent for the loss in temperature occasioned by distance from the equator? As an instance of this difference, Fort Simpson in latitude 62° N. has, between May 1 and August 31, 342 hours of daylight in excess of that received at Ottawa in latitude 45.5° N. A similar condition as regards light holds good on the higher slopes of mountains.

PFEFFER, in his *Physiology of Plants* (Engl. transl., **2**: p. 98) writes as follows:

> Plants are able to grow when continuously illuminated both in the polar regions and under artificial conditions, but the future must show whether all plants grow normally under light of constant intensity. For various reasons the same total quantity of light will not produce the same physiological effects when spread over the entire twenty-four hours, as when restricted to twelve hours of the day, or an even shorter period.

To determine the effect of shading plants during a short period each day at the time of year when the sun is longest above the horizon, certain experiments were made in 1916 at Ottawa, and repeated again during 1918. *Linum usitatissimum* was chosen, for the reason that it is shallow rooting, and has little tendency to branch when sown moderately thick, both of which are important considerations when plants have to be grown in pots.

The procedure followed in 1916 differed considerably from that of 1918, and will be described first. Four pots, each being 10 inches in diameter, were filled with the same kind of soil and were sunk in the

open ground up to the brim. A bell jar of about the same diameter as the pots was covered with brown paper and was placed over one of the pots for a certain time during the morning and over another during the afternoon, the exact duration of shading being noted in each case. The other two pots were left as checks. Care was taken to place the bell jar over the plants as far as possible during the time when no rain was falling, as the presence of additional moisture in some of the pots would have caused a disturbing factor in estimating the results. The temperature inside the bell jar was not noted, but there is no reason to believe that it was so different as to have any marked effect.

The pots were labeled *A*, *B*, *C*, and *D;* of these *A* and *C* were shaded, while *B* and *D* were the check pots. The seeds were sown in the pots on June 13, 1916. On June 26, when the first leaf in succession to the cotyledons had developed, 80 seedlings were left in each pot; the others in excess of this number were pulled up and thrown away.

Pots *A* and *C* were shaded on 11 days, beginning with June 26 and ending with July 10, the total duration of darkening being 28 hours and 23 minutes, in the case of pot *A;* and 28 hours and 28 minutes in pot *C*, *A* being shaded before midday and *C* in the afternoon.

On July 11 the number of plants in each pot was 64 in *A*, 68 in *B*, 78 in *C*, and 63 in *D*. On this date five of the tallest plants in each pot were pulled up, measured, and weighed, after shaking the earth from the roots. The average length and weight per plant in each lot were as follows:

Pot	Length	Weight
A (darkened)	109.2 mm.	122 mg.
B	112.4	125
C (darkened)	111.2	121
D	125.8	212

From July 12 to 20 inclusive, pots *A* and *C* were again shaded daily, except on the 16th, and the total period of darkening was 17 hours and 1 minute. On July 22 the 10 tallest plants were selected from each pot, measured, and weighed, with the following results per plant:

Pot	Length	Weight
A (darkened)	206.5 mm.	478 mg.
B	238.2	572
C (darkened)	183.5	430
D	256.5	936

On July 31 the first flowers were opening in pots *B* and *D*, while the first flower opened in pot *A* on August 2, and in pot *C* on August 4.

On August 10 ten of the tallest plants remaining in each pot were pulled up and weighed, with the following average results per plant:

Pot	Weight
A (darkened)	852 mg.
B	1160
C (darkened)	822
D	1712

These were the last observations made during 1916; no opportunity occurred to continue the work in 1917. In 1918 four 10-inch pots were again used, those labeled *A* and *C* being darkened, while *B* and *D* remained uncovered. As pots *A* and *B* were sown and examined on different dates from *C* and *D*, the data for each pair will be given separately. In both sets the pots were darkened during the morning only; on two out of every three days on which the plants were shaded the sun was shining at the time.

Pots *A* and *B* were sown with flax on May 7. On June 12, 29 of the tallest plants were left in each pot, the others being removed. Pot *A* was darkened altogether 34 hours and 53 minutes on 15 days, from June 12 to 29. On July 2 nine plants were in flower in each pot, and seeds were ripe in each on July 25. On July 27 all the plants were pulled up, measured, and weighed with the following results:

Pot	Average length	Average weight	Average number of capsules per plant
A (darkened)	514.6 mm.	1136 mg.	4.6
B	526.0	1236	5.5

Pots *C* and *D* were sown on June 8. On July 4, 36 plants were left in each pot. Pot *C* was darkened for 43 hours and 30 minutes on 18 days, from July 4 to 25. On July 25 two plants were in flower in *C* and six in *D*, while in both the first seeds were ripe on August 15. On this date the ten best plants in each pot were pulled up, measured, and weighed. The results are as follows:

Pot	Average length	Average weight	Average number of capsules per plant
C (darkened)	431.5 mm.	1248 mg.	5.0
D	530.5	2397	6.5

Summary and conclusions

1. Flax plants were raised in 10-inch pots placed in the open and sunk in the ground up to the brim.

2. From certain pots the light was excluded for 2–2.5 hours per day during periods of 11–19 days in all.

3. The shading of the plants took place between June 12 and July 25, and occurred at or near the time of year when the amount of daylight was greatest.

4. Tests were made on the shaded and unshaded plants as regards (1) average height, (2) average weight, and (3) average number of capsules produced. In every case the unshaded plants gave a higher figure.

5. As stems grow in length at a more rapid rate in darkness than in light, it might have been expected that the average height of the darkened plants would at least have equaled that of the unshaded ones, but the contrary was the case.—J. ADAMS, *Central Experimental Farm, Ottawa, Canada.*

PIER ANDREA SACCARDO

Dr. PIER ANDREA SACCARDO, who died February 12, 1920, was born at Treviso, Italy, in 1845. At the age of 21 he became connected with the Botanic Garden in Padua, where he remained until his death, first as Assistant Director, then as Director (1878) and Professor of Botany in the Royal University of Padua. He gave especial attention to Fungi, and contributed many papers to mycological literature. Among them were *Fungi Veneti novi vel critici*, series I–XII (1873–1882), and *Notae Mycologicae*, series I–XX (1890–1916). In the latter were included descriptions of new species from various regions of North America and from South America. He also published *Fungi Italici autographice delineati* (pls. 1–1280), and issued a set of *exsiccati* under the title *Mycothecae venetae* (cent. I–XI), and was editor and principal contributor to the mycological journal *Michelia*.

When SACCARDO began his labors in mycology, the general works of PERSOON and of FRIES had become antiquated. New systems of classification had been proposed, and descriptions of new genera and species had appeared in publications treating of limited regions and scattered in periodical literature and society transactions. The fame of SACCARDO will rest most largely on the measures he took to meet this situation. He projected and carried through the publication, in one series, of descriptions of all known species of Fungi based in the beginning on 600 separate publications. The first volume of this work (*Sylloge Fungorum omnium hucusque cognitorum*) appeared in 1882, and the task was finished when vol. VIII appeared at the end of 1889, the volumes

averaging about 600 pages each and being provided with keys and adequate indexes to genera, species, and hosts. With the publication of vol. IV he found it necessary to have assistance, and in subsequent volumes CUBONI, MANCINI, BERLESE, DETONI, ED. FISCHER, PAOLETTI, and TREVISAN collaborated. To carry out this plan, however, there was first of all necessary a general system of classification, and the one which he devised and used in the *Sylloge* has been followed in practically all subsequent work in mycology. That it was a natural and perfectly satisfactory one no one would affirm, but it was perhaps the best that could be framed in the present state of knowledge of the Fungi, and will probably remain in use as a practical, workable system until such time as a more natural one can be devised.

The completion of this work did not end SACCARDO's labors. Mycological activity, stimulated by the publication of the *Sylloge*, was such that it quickly became necessary to issue supplementary volumes, the first of which appeared in 1891, the last, a work of 1600 pages, bringing the matter up to the end of 1910, in 1913. P. SYDOW, MUSSAT, D. SACCARDO, TRAVERSO, and TROTTER were collaborators in the preparation of these supplementary volumes, which included 2 volumes (2467 pages) of index to published figures of Fungi. The 22 volumes of the *Sylloge Fungorum* constitute the working handbook of every mycologist.

SACCARDO was a good correspondent and a gracious one. Material was sent to him from all quarters for determination, and he became the court of last resort to many mycologists, and in this way as well as through his publications he gave impetus to the study of Fungi. The effect of his work illustrates again the fact that progress in botany, as in other sciences, is based not only on brilliant research and broad generalization, but also on a large amount of downright drudgery.—J. J. DAVIS, *University of Wisconsin, Madison, Wisconsin.*

CURRENT LITERATURE

BOOK REVIEWS

Hardwoods of Australia

Ten years ago BAKER[1] published an elaborate book on the pines of Australia. He immediately commenced work upon a companion volume dealing with the native hardwoods, and some of the material was on exhibition at the Sydney Technological Museum, of which Dr. BAKER has long been the director. The work has now been completed, and it brings great credit, not only to the author,[2] but to the Museum and Commonwealth for undertaking and financing an unusually expensive investigation.

The dominant object of the work is to make known to Australians, and to the world in general, the diversity and economic value of the Australian hardwoods. In America, and doubtless in many other countries, the popular mind has become so accustomed to mahogany as the conventional wood for pianos, victrolas, and fine furniture, that other woods, which might be stronger and more beautiful, receive scant recognition.

BAKER has had various kinds of plain and ornamental furniture, plain and carved interior furnishings, and a great variety of useful and ornamental things, from carved gavels to railway bridges, made from native woods, and has shown conclusively that Australians do not need to go outside their own country for any kind of timber. The needs of the architect, builder, engineer, cabinet-maker, and forester are kept in mind, and valuable suggestions, based upon practical experiments, form a feature of the work.

It is interesting to learn that not less than nine-tenths of the Australian trees are hardwoods. The United States and Canada have about 700 species of trees; Australia has about 500, but many of them have a wide range. The genus with the largest number of species, the greatest variety in hardness, color, and finish, as well as the widest distribution, is *Eucalyptus;* and in reforestation the genus could hardly be surpassed, for the blue gum in 24 years becomes as large as the English oak in 200 years. One figure of *Eucalyptus regnans* shows annual rings with a width of more than a centimeter. This is not cited as a record, for the author remarks that a specimen of *Sequoia sempervirens* at Reefton, New Zealand, known to be 27 years old, was "nearly 3 ft. in diameter, with some of the rings measuring an inch in width." This means that reforestation would be so rapid that it would have an immediate practical aspect. Species of *Acacia* yield extremely hard timber, some as red

[1] BAKER, RICHARD I., and SMITH, HENRY G., A research on the pines of Australia. 4to. pp. xvi+548. Sydney. Government of New South Wales. 1910.

[2] BAKER, RICHARD T., The hardwoods of Australia and their economics. 4to. pp. xvi+523. Sydney. The Government of New South Wales. 1919. £1. 5s.

as rosewood, some as black as ebony, while others have a lighter color. Species of *Casuarina* also yield hard wood of various colors, some looking like oak already fumed.

The hardness and weight of *Eucalyptus* timbers are due to the predominance of thick-walled fibers. In some of the extremely hard woods of the genus the vessels are almost entirely blocked by tyloses. The figure in the wood is not due to large rays, as in oaks, but to the fact that fibers and wood elements run in waves. In color, this single genus furnishes perfect imitations of maple, locust, cherry, mahogany, and rosewood; while the timber, as hard and strong as any of these, takes a magnificent polish.

The taxonomic sequence follows that of BENTHAM and HOOKER. In each case there is a systematic diagnosis, with geographical range, and a description of the timber and its uses. Local names are given in addition to the scientific names. The rank in a scale of hardness and the weight per cubic foot are also given, and some of this information is summed up in a table according to hardness: extremely hard, very hard, hard, and moderate. In most cases photomicrographs illustrate transverse, longitudinal radial, and longitudinal tangential sections, which not only show the structure but also indicate the strength, hardness, and weight of the wood.

An unusual feature is a table of combustibility. Since wood in Australia is used to a considerable extent in railroad bridges and in shipbuilding, resistance to fire is a very desirable quality. By means of a "xylopyre" the time required to burn up a piece of wood of a definite size was determined with great accuracy. These tests show that many of the Australian woods are remarkably resistant to fire. In this quality *Eucalyptus Fletcheri* easily heads the list, with 19 minutes required to burn the test piece; next comes *Syncarpia laurifolia*, with 12 minutes; then *Casuarina torulosa*, with 8 minutes; followed by many species of *Eucalyptus* ranging from 7 minutes down to 3 minutes. The significance is evident when we note that in the same test our *Pseudotsuga Douglasii* has a time limit of 4 minutes, *Quercus alba* 3 minutes, *Juglans* sp., *Fagus sylvatica*, and *Sequoia sempervirens* less than 3 minutes.

A striking feature of the work, and one most likely to give it immediate practical importance, is a series of 126 magnificent plates in color, illustrating the natural appearance of the wood. These plates, together with the photographs of various articles, inside furnishings, buildings, etc., prove the variety and value of the Australian hardwoods.

The timely warnings, calling attention to the desirability of sane lumbering methods and the necessity for reforestation, should be heeded while the timber supply is still abundant.—C. J. CHAMBERLAIN.

NOTES FOR STUDENTS

Form and growth of trees.—In 1913 the Schnyder von Wartensee Foundation opened a prize competition of three years' duration "to stimulate new investigations upon the growth in thickness of trees." First prizes

subsequently were awarded to ARNOLD ENGLER and PAUL JACCARD, professors in the Federal Polytechnical School in Zurich.

ENGLER[3] concerns himself with the effects of geotropic and heliotropic stimuli upon the form and structure of arborescent plants. He is of the opinion that old stout stems and branches of dicotyledons may develop marked geotropic and heliotropic curvatures, but considers that, in the case of the Coniferae, heliotropic bending is confined to the younger, more pliable portions of the stem. He devotes considerable attention to the study of the form and growth of broad-leaved trees on steep slopes, and concludes that the terminal shoots, particularly during the earlier stages in the ontogenetic development of the trees, tend to bend downhill toward more intensive illumination upon that side. During subsequent growth these curvatures are more or less completely neutralized by bending in the opposite direction in response to geotropic stimuli. His numerous stem analyses show that trees growing on steep slopes may be eccentric on the uphill side, the downhill side, or vary in their eccentricity at succeeding heights in the stem. Accelerated growth upon the uphill side is assumed to be due to geotropic stimuli, regardless of whether the stem is concave or convex, and eccentricity on the downhill side, as in Coniferae, to longitudinal compression upon the cambial layer. He reaches similar conclusions in regard to the eccentricities of stems and branches of trees growing upon level ground. In other words, accelerated growth upon the upper sides of stems or branches is geotropic, whereas that upon the under sides is due to longitudinal compression. The geotropic stimulus ceases to act only when the terminal shoot occupies a vertical position. Different parts of a tree may react differently toward light and gravitational forces. Thus in the younger (higher) portions of a stem heliotropic frequently overshadow geotropic stimuli, so that geotropic curvature and eccentricity are confined to the base of the stem. In dicotyledons the influence of gravity usually exceeds that of longitudinal compression, and accelerated growth of the under sides of stems and branches is found only where it is not inhibited by negative geotropism. Lateral eccentricity occurs when these two factors, working in opposition, neutralize each other. Longitudinal compression affects the volume of secondary xylem but not its structure. In ring-porous dicotyledons, "geotropic wood" is characterized by wider vessels and a greater proportion of summer wood; but in diffuse porous species, the wood of the upper and lower sides of stems and branches is of the same general type.

As evidence in favor of these views, ENGLER cites the crooked or curved stems of trees growing under peculiar environmental conditions; for example, on steep slopes, displaced from the normal vertical position, in unilateral

[3] Tropismen und exzentrisches Dickenwachstum der Bäume; Ein Beitrag zur Physiologie und Morphologie der Holzgewächse. Schr. Stift. Schnyder von Wartensee Zürich **21**:1–106. *figs.* 1–30. 1918.

illumination, etc.; such data as may be secured by critical field observations, detailed stem analyses, and a careful study of the past environmental history of the plants. Thus the method of attacking the problem consists in showing that variations in form are closely correlated in each case with variations in illumination, gravitational, or mechanical forces. Since it places a premium upon circumstantial evidence and deductive reasoning, its ultimate success is dependent upon the number of concordant facts that can be advanced in its favor. Although ENGLER's data indicate that bending actually occurs in old stems, they do not demonstrate in all cases that the curvatures are due necessarily to particular stimuli. For example, in tall dense forests, the sudden curvatures of trees toward gaps made by thinnings may be heliotropic; but it is also conceivable that they may be due to a lack of rigidity in tall (13–18 m.) very slender (8–13 cm.) trees. The reviewer has seen thinnings in lodgepole pine forests in which the crowns of tall unsupported trees have bent over until they touched the ground. Furthermore, if bending occurs in response to heliotropic stimuli and is produced by the activity of living cells (parenchyma) in the sapwood, as ENGLER supposes, the structure of the stem must be considerably modified. No conclusive evidence is presented to indicate that such modifications actually have taken place.

In the second of the prize essays, JACCARD[4] attacks the problem of the form and growth of trees from an entirely different angle. The first twelve chapters of his memoir are devoted to a criticism of the SCHWENDENER-METZGER hypothesis, which holds that the form of trees is determined largely by mechanical factors (wind and gravity), and to an exposition of his own theory that the "clear length" of the stem is, at successive heights, a shaft of equal water-conducting capacity. Inasmuch as this portion of the memoir is largely a recapitulation of former papers, which have been reviewed by GROSSENBACHER[5] and others, it may be passed over without further comment. The three succeeding chapters (pp. 101–169) are concerned with interesting experiments, designed to test the effects of mechanical, geotropic, and heliotropic stimuli and various types of girdling upon the form and anatomical structure of conifers and dicotyledons. A large number of young stems and branches were subjected to various types of flexure (sustained or intermittent). Their subsequent growth, form, and structure were found to vary, depending upon the intensity and duration of the stimuli. Thus if the stem of an erect conifer is bent alternately to the north and south, no "redwood" is formed unless the stem is allowed to remain in each posture for a certain period

[4] JACCARD, PAUL, Nouvelles recherches sur l'accroissement en épaisseur des arbres: Essai d'une théorie physiologique de leur croissance concentrique et excentrique. Pub. Foundation Schnyder von Wartensee Zürich **23**: i–xii+1–200. *pls. 1–32. figs. 1–75.* 1919.

[5] GROSSENBACHER, J. G., The periodicity and distribution of radial growth in trees and their relation to the development of "annual" rings. Trans. Wis. Acad. Sci. **18**: part 1. 1915.

of time. Again, a slight curvature may accelerate the growth on the upper side of a dicotyledonous stem, whereas a more pronounced bend may produce eccentricity upon the under side or inhibit the growth of both the upper and under sides and lead to lateral eccentricity.

In the fifth and concluding section of the volume, JACCARD elaborates the following hypothesis: "The morphological characters common to all trees are determined (1) by the polarity of their organs, that is to say, by their tendency to grow most rapidly in a vertical direction, and (2) by the modifications which the exigencies of nutrition and the action of external forces (gravity, heat, light) impress upon this polarity. These modifications manifest themselves through the osmotic force of cells which engenders, on the one hand, two circulatory currents (the ascending sap and descending current of elaborated substances), and, on the other hand, mechanical strains and stresses (pressure of turgescence) capable of influencing the form of cells. . . . . In general, such variations in gross form and anatomical structure, as may be observed at different levels in the concentric, vertical axes of trees, are determined by the physical conditions of the transpiration stream and the flow of elaborated sap. On the contrary, the anatomical differentiation and variations in transverse sections, which are concomitants of the eccentric growth of inclined or horizontal branches, are due to mechanical forces engendered by the unequally rapid growth of the antagonistic sides of these organs, under the asymmetrical influence of gravity and light."

Although the author is justified in contending that the problem of the growth and form of stems and branches should be attacked from the point of view of fundamental physiological phenomena, and in rejecting teleological conclusions as unscientific, he extends his own generalizations much farther than is warranted by his experimental data. When one considers how little is actually known about the "ascent of sap," the growth and activities of the cambium and its derivative tissues, the distribution of food substances and osmotic pressures, and, in general, concerning transpiration, metabolism, and translocation and their interactivities in arborescent plants, one is inclined to question whether there are available at present sufficient reliable data to form the basis for such a comprehensive hypothesis as is formulated by the author.—I. W. BAILEY.

Factors of fruitfulness.—A contribution by WIGGINS[6] covers investigations for 5 years, chiefly upon trees that were 8 years old at the beginning of the experiment in 1913. Attention was centered upon the individual fruiting branch "in an effort to determine the effect of certain conditions and practices upon the development and performance of the individual fruit spur."

The data for the performance of the individual spurs were obtained from 8-year-old Rome, Gano, Winesap, Grimes, York, and Jonathan. The first selection was made of fruiting spurs, but after that a blossoming spur was

[6] WIGGINS, C. C., Mo. Exp. Sta. Research Bull. no. 32. 1–60. 1918.

considered to be a fruiting spur for the season in which it bloomed. It might be questioned whether the author was justified in making such an assumption.

Jonathan, Winesap, and Grimes were found to produce a fair amount of bloom each year, but with no exceedingly productive seasons; while Rome, York, and Gano were found to have a very high percentage of bloom one season and a comparatively low one the next. Winesap and Jonathan, in the order named, were able to develop blossoms in successive seasons on the same spur in a much greater proportion than the other varieties. The difference between alternating and non-alternating varieties seems to be due to the ability of the spurs of the regular bearing kinds to blossom two years in succession. The most effective fruiting age for the spurs, irrespective of the type of bearing, appeared to be from 3 to 7 years.

Determinations of the relative amounts of food reserve in the fruit spurs were made by finding the lowering of the freezing point in the spur sap by means of a Beckman apparatus. Only relative proportions of reserves were indicated by the lowering of the freezing point, and starch not at all. It was found that sap from the bearing spurs had a slightly higher concentration during a considerable portion of the year than sap from non-bearing spurs. It must be remembered, however, that the method of analysis gave no indication of the amount of starch that might have been present. It was found that during the latter part of June and early July there was a sudden drop in concentration of sap, both in fruiting spurs and non-fruiting spurs, and at that time both kinds of spurs reached a similar degree of concentration. The author concluded also that little difference in concentration of the cell sap could be attributed to soil conditions or to the number of fruits being produced upon a spur.

Chemical analysis of the spurs of Yellow Transparent were made in order to ascertain the amounts of sugars and starch stored. It was found, in the majority of cases, that there was a slightly greater amount of sugar, both reducing and total, in the non-bearing spurs. The starch content of the non-bearing spurs did not average quite so high as that of the bearing spurs, but there was considerable variation in these results. Inasmuch as the determinations made were comparatively few in number (22) and covered only the period from late October to April, no conclusions were drawn. A determination of nitrogen would have made this work much more valuable.

It was found that non-bearing spurs had a larger total leaf area than bearing spurs, and that the difference was due more to the number of leaves than to the size of the individual leaves.

The effects of girdling upon the concentration of cell sap were determined by noting the depression of the freezing point. The sap of all parts above the girdle was found to have an increased concentration, and the sap of all parts below the girdle a decreased density when compared with sap from corresponding parts of similar but ungirdled trees. The greatest effect was observed upon the sap of the trunk, and the difference became less as the distance from

the girdle increased; consequently the leaves and twigs at the periphery of the tree, where the majority of the fruit buds were formed, did not show such a great variation.

The experiment to determine the effect of fertilizers upon fruitfulness was started with 1-year-old Rome on Paradise stock. The trees were planted in separate large wooden tubs, half of them filled with Missouri River sand and half with loess soil. The fertilizers were applied just as growth was beginning in the spring. It was found that nitrogen was a very decisive factor in the growth of the tree, the development of fruiting wood, and the formation of blossoms. Phosphorus and potassium, either singly or in combination, had no apparent effects.

The effect of various systems of soil management was noted upon the concentration of the cell sap. There were 5 different cultural plots: clean cultivation with soy beans or cow peas planted in June; successive crops of corn; seeded to red clover in alternate years; successive cropping of alfalfa; and permanent timothy sod. "These experiments showed conclusively that tillage methods materially affected the sap density of the twigs of the apple tree." The plots ranked in sap concentration of the twigs as follows: alfalfa; timothy and blue grass sod; clover; corn; and clean cultivation with legume cover planted in June. There was very little difference between the clover and corn plots. The trees in the most intensively cultivated areas were considerably the largest.

A group of 64 one-year-old Delicious trees were used to study the effect of pruning methods upon the formation of fruiting parts. During the first 3 years the trees headed at 2 feet made a greater amount of twig growth and produced a larger number of short branches or potential fruiting wood than did the trees headed at 5 feet. Each month a separate 5- to 6-year-old Jonathan tree was subjected to etherization. Very little effect was observed upon the concentration of the sap of the spurs or of the leaves, and the small difference noted appeared to be only temporary. A rather extensive bibliography accompanies the article.—H. W. RICHEY.

Phylogeny of seed plants.—At the St. Louis meeting of the American Association, the botanical program included a symposium on the phylogeny of seed plants. The three invitation papers have just been published. The three investigators, working upon different phases of the problem, have shown tendencies in the evolution of the groups with which they are concerned; and while the phylogeny of seed plants still represents a great field for exploration, some results have been obtained, and the problem has been advanced a little toward the distant solution.

BUCHHOLZ[7] has made a comprehensive survey of the development of the embryo and polyembryony in the conifers, much of the subject-matter being

[7] BUCHHOLZ, J. T., Embryo development and polyembryony in relation to the phylogeny of conifers. Amer. Jour. Bot. 7:125–145. 1920.

his own contribution. He concludes that the apical cell in early embryogeny, cleavage polyembryony, rosette embryos, rosette cells, and the direct organization of embryo initials from free nuclei of the proembryo are primitive features; while the organization of embryo initials after walls form in the proembryo, a proembryo that fills the entire egg with cells, the archegonium complex, the embryo cap, and the return to simple polyembrony are advanced or specialized features. A study of polyembryony throughout the groups shows that cleavage polyembryony tends to become more or less eliminated in passing from the lower to the higher genera, and consequently the conifers must have been derived from ferns with cleavage polyembryony.

CHAMBERLAIN,[8] in dealing with the Cycads, considered two questions: "What has been their origin?" and "Have they left any progeny?" From a study of the comparative morphology of the entire Cycadophyte phylum, from the Paleozoic to the present time, he concludes that the Cycads could not have come from any Mesozoic forms of the *Cycadeoidea* type, or from any known forms of the Lower Mesozoic. If they have come from any of the Bennettitales, they have come from forms so nearly like the Cycadofilicales, to which the Bennettitales themselves owe their origin, that whether the Cycads are an early branch from the Bennettitales, or have come from the Cycadofilicales directly, can be answered only by fossils still to be discovered and studied.

The second question, so far as the living Cycads are concerned, is answered positively in the negative. The groups of living seed plants are considered separately, and the conclusion reached that the Cycads are not responsible for any of them. This conclusion was emphasized by some facts indicating that the Coniferophytes and Angiosperms have a more reasonable origin in the Ferns or Lycopods. Stress is laid upon the fact that the extinct forms which have been preserved are mostly woody, especially in the Mesozoic. Have herbaceous Gymnosperms been lost which may have given rise to herbaceous Angiosperms? Could such Angiosperms have given rise to the woody Angiosperms which became prominent in the Cretaceous? It is difficult to derive these Angiosperms from any known woody Gymnosperms.

The general conclusion is that the Cycadophytes have come from Ferns, and that they have not left any progeny outside of the Cycadophyte line.

WIELAND[9] deals first with the distribution of seed plants, almost exclusively with fossil seed plants, and then discusses relationships. In living plants, only lateral distribution is considered, but in fossil forms both lateral and vertical distribution must be studied. The vertical distribution, in most cases, is better known than the lateral, and the period of extinction is more determinable than the first appearance. The Carboniferous flora is better

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[8] CHAMBERLAIN, C. J., The living Cycads and the phylogeny of seed plants. Amer. Jour. Bot. 7:146–153. 1920.

[9] WIELAND, G. R., Distribution and relationships of the Cycadeoids. Amer. Jour. Bot. 7:154–171. 1920.

known because the economic value of coal has uncovered immense areas; while the Permian, Rhaetic, or Middle Triassic have depended upon the enthusiasm of about a dozen scientists. The flora of these horizons is probably as abundant and varied as that of the Carboniferous, but not so available.

In going back through the geological horizons, there is a gradual merging of Coniferophyte, Cycadophyte, and Ginkgophyte foliage toward seed-bearing "quasi-ferns." Also toward the early Paleozoic there seems to be some kind of contact between the early seed ferns and the older Lepidophyte types leading toward the primitive Gymnosperms. Whether well down in the Devonian some of the Lepidophytes, like the later seed ferns, may also have led into the primitive Gymnosperms is the real riddle of paleobotany, more so than the origin of Angiosperms. In almost all instances the doubtful border of Cycadeoid foliage ends in a tree forest of seed ferns, *Cordaites*, pines, araucarians, and Ginkgoes, but never in a recognizable scrub. It is stated that among the Cycadeoids will be found the lost forests and the greatest forest makers of the Mesozoic.

WIELAND suggests that from age to age great groups have come down side by side, undergoing endless change and losing apparent relationships; but almost no forms, scarcely a family, need be regarded as more ancient or more modern than any other. It is conceivable that all the antecedent types of Angiosperms are discrete separate lines leading back to the first forests of the Devonian.—J. M. C.

History of cotyledony.—BUCHHOLZ,[10] in connection with his studies of embryo development in conifers, has reached certain conclusions in reference to the primitive condition of cotyledony and its subsequent evolution. His investigations showed that in a number of conifers fusions of cotyledons occur during embryogeny, and that there is no evidence of splitting. Fusion results not merely in a reduced number of cotyledons, but often in the development of cotyledonary tubes. The conclusion is that the primitive gymnosperm embryo had numerous cotyledons; that fusions resulted in a reduced number; that dicotyledony was attained either by a fusion of cotyledons into two groups or by an extremely bilabiate development of a cotyledonary tube; and that monocotyledony is the result of a cotyledonary tube becoming "unilabiate" in its development. According to these conclusions, therefore, polycotyledony is primitive, dicotyledony is derived, and monocotyledony is the extreme expression of cotyledonary fusion.—J. M. C.

Life cycle of climbing bamboo.—SEIFRIZ[11] has published some observations on one of the climbing bamboos (*Chusquea abietifolia*) growing in Jamaica.

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[10] BUCHHOLZ, J. T., Studies concerning the evolutionary status of polycotyledony. Amer. Jour. Bot. **6**:106–119. *figs. 25*. 1919.

[11] SEIFRIZ, W., The length of the life cycle of a climbing bamboo; a striking case of sexual periodicity in *Chusquea abietifolia* Griseb. Amer. Jour. Bot. **7**:83–94. *figs. 5*. 1920.

It is a species little known outside Jamaica, and is restricted there to the mountainous interior. It is one of the plants that live vegetatively for a number of years and then flower and die. *Agave americana* ("century plant") is the most commonly cited illustration of this habit. The bamboos are notable for this kind of periodicity, the number of years of vegetative activity before flowering varying widely in different forms. Apparently, when flowering occurs, most of the individuals of a region are involved, and presently all the mature plants are dead, and the ground occupied by seedlings. SEIFRIZ had an opportunity to observe the flowering condition of *Chusquea abietifolia* in 1918, and the records available showed that the previous flowering condition had occurred 33 years before. The explanation of this behavior is not available as yet, for seasonal factors controlling such long periods are very unlikely.—J. M. C.

Mosaic disease of spinach.—Investigations of JODIDI, MOULTON, and MARKLEY,[12] of the Bureau of Plant Industry, have shown that "spinach plants, especially their tops, affected with mosaic disease, have a smaller percentage of total nitrate, acid amide, mono and diamino nitrogen, but a somewhat larger percentage of ammonia than normal plants, nitrous acid being present in diseased plants only. This is due to the fact that denitrification takes place whereby nitrates are reduced to nitrites which, reacting on various nitrogenous compounds present in the spinach, bring about elimination of nitrogen in a free state, involving also loss of nitrogen in the form of ammonia." It will be very interesting to know how generally in physiological diseases of plants and in viris and other disorders denitrification is involved.[13]—WM. CROCKER.

Leaching of nitrates.—Working with uncropped and unmanured soils RUSSELL and RICHARDS[14] conclude that "the nitrate in drainage water accounts for practically all the nitrogen lost from the soil. The uncertainty attaching to the estimated figures and to the actual amount of new nitrogen in the rainfall deprives the balance sheet of precision, but there is no room for much fixation or loss of gaseous nitrogen. The chief, if not the sole action, in this soil when there is no manure, crop residues, or fresh supply of organic matter, is the production of nitrate. It is in these circumstances that the nitrogen cycle is seen at its simplest. We know from other Rothamsted experiments that the cycle becomes more complex when organic matter is added to the soil, both fixation and loss of nitrogen being then liable to occur."—WM. CROCKER.

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[12] JODIDI, S. L., MOULTON, S. C., and MARKLEY, K. S., The mosaic disease of spinach as characterized by its nitrogen constituents. Jour. Am. Chem. Soc. **42**:1061–1070. 1920.

[13] BOT. GAZ. **65**:199–200. 1918.

[14] RUSSELL, E. J., and RICHARDS, E. H., The washing out of nitrates by drainage water from uncropped and unmanured land. Jour. Agric. Sci. **10**:22–43. 1919.

Contraction of roots.—Miss CHURCH[15] has studied the contraction of roots, and while she thinks some of RIMBACH's conclusions were not justified by his facts, she thinks the following can be accepted: "(1) roots do shorten; (2) the parenchymous tissues of the root are the seat of activity; (3) the cork and the vascular trace are passive; (4) the cork is ultimately crushed; (5) there is a region where one can see wrinkling and measure shortening, a second region where no wrinklings are visible yet one can measure shortening, and an unchanged region (RIMBACH); in dicotyledons the trace becomes visibly curved inward and outward in a wavy fashion, while in monocotyledons the vascular bundles remain practically straight (DEVRIES)."—WM. CROCKER.

Energy of biological processes.—LINHART[16], in a preliminary paper, has called attention to the almost neglected field of study of the energy relations in biological processes. By employing similar methods to those recently used in attempts to base chemical reactions on thermodynamic principles, LINHART hopes to be able to measure the energy values of various biological processes. The heats of combustion of the nutrient materials must be known. With these data, and the entropies of the substances involved, it is possible by thermodynamic equations to compute the free energy from a reaction. The energy available to *Azotobacter* grown on mannite was thus calculated. The amount of NH_3 fixed by *Azotobacter* in consuming a certain amount of mannite was found to represent only about 1 per cent of the energy value of the mannite.—J. R. MAGNESS.

Heat treatment of seeds.—ATANASOFF and JOHNSON[17] give a preliminary report of their work on heating cereal seeds as a means of killing seed-carried parasites. They emphasize the necessity of applying this method only to high quality, well dried seeds. They could thus dispose of bacterial blight of barley and of oats. The wheat scab (*Gibberella soubinetii* and *Fusarium* spp.), primary infections only, and spot blotch of barley (*Helminthosporium sativum*) are practically eliminated by dry heat treatment. The *Helminthosporium* blotch of oats, as well as loose smut of barley and smuts of oats, were markedly reduced by such treatment. In all of these cases, of course, the germination was not materially injured.—WM. CROCKER.

Soil moisture.—KEEN[18] gives an excellent discussion of the latest literature on the mechanics of soil moisture.—WM. CROCKER.

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[15] CHURCH, MARGARET B., Root contraction. Plant World **22**:337–340. 1919.

[16] LINHART, G. A., The free energy of biological processes. Jour. Gen. Physiology. V. **2**:247–251. 1920.

[17] ATANASOFF, D., and JOHNSON, A. G., Treatment of cereal seeds by dry heat. Jour. Agric. Res. **18**:379–390. 1920.

[18] KEEN, B. A., The relation existing between the soil and its water content. Jour. Agric. Sci. **10**:44–71. 1919.

VOLUME LXX NUMBER 3

Bl # 36904

THE BOTANICAL GAZETTE

SEPTEMBER 1920

NEW SPECIES OF PIPER FROM CENTRAL AMERICA[1]

CASIMIR DE CANDOLLE

Piper reptabundum, n.sp.—Ramulis glabris; foliis brevissime petiolatis glabris, limbo oblique oblongo inaequilatero, basi inaequilatera altero latere rotundato altero obtuso apice longe acuminato, nervo centrali alte ultra medium nervos adscendentes utrinque 5 mittente, petiolo glabro basi ima vaginante; pedunculo glabro petiolum pluries superante filiformi; spica quam limbi dimidium multo breviore et filiformi, bracteae pelta triangulari glabra et glandulis conspersa, pedicello lato puberulo, filamentis exsertis rhachi ad basin baccae insertis, bacca libera trigona vertice glandulis conspersa, stigmatibus linearibus.

Repens vel scandens. Ramuli e nodis radicantes circiter 1.5 mm. crassi, collenchyma continuum libriforme, fasciculi intramedullares 1-seriati pauci. Limbi in sicco membranacei pellucido-punctulati usque ad 14.5 cm. longi, in medio a nervo centrali altero latere usque ad 2 cm. altero usque ad 3 cm. lati. Petioli usque ad limbi latus longius vix 2 mm., inter latera 2 mm. longi. Pedunculi fere 5 mm. longi. Stamina 3. Stigmata 3 sessilia.

COSTA RICA: Forêts de Shirores, Talamanca, alt. 1000 m., *H. Pittier* 9277.

Piper purulhanum, n.sp.—Ramulis glabris; foliis modice petiolatis glabris, limbo ovato-acuminato basi ima aequilatera

[1] The descriptions of new species presented herewith are taken from an extended manuscript account of the Panamanian and Central American species of *Piper* prepared by the late CASIMIR DE CANDOLLE, largely upon material in the U.S. National Herbarium, the Central American specimens having been included in material sent to M. DE CANDOLLE for elaboration in connection with his study of the Piperaceae collected during the recent biological survey of Panama under the auspices of the Smithsonian Institution.—WILLIAM R. MAXON, U.S. National Museum.

acuto apice obtusiuscule acuminato, nervo centrali nervos adscendentes utrinque 2 quorum supremus a 2–3 cm. supra basin solutus nervulosque validos 2–3 adscendentes mittente, nervo laterali adscendente utrinque a basi soluto, petiolo basi ima vaginante; pedunculo glabro quam petiolus breviore, spica quam limbus paullo breviore apice acuta, rhachi hirsuta, bractea spathulata apice triangulari cucullata et glabra inferne margine hirsuta, antheris oblong-ellipticis quam filamenta oblonga paullo breviori-bus, ovario libero glabro, stigmatibus linearibus.

Ramuli spiciferi 2.5 mm. crassi, collenchyma fere continuum libriforme, fasciculi intramedullares 1-seriati, cellulae sclerosae in cortice circa collenchyma crebrae, canalis lysigenus unicus centralis. Limbi in sicco firmi minute pellucido-punctulati usque ad 17 cm. longi et 10.5 cm. lati. Petioli 25 mm., pedunculi 8 mm. longi. Spicae florentes 14 cm. longae et 3.5 mm. crassae, canali lysigeno centrali munitae. Stamina 3 rhachi ad basin ovarii inserta, antherae paullo sub 1 mm. longae. Stigmata 3 sessilia.

GUATEMALA: Dept. Baja Verapaz, forest near Purulhá, alt. 1800 m., *H. von Türckheim* II. 1705.

Piper tenuispicum, n.sp.—Ramulis glabris; foliis brevissime petiolatis glabris, limbo elliptico-oblongo basi subaequilatera acuto apice longe acuminato acumine mucronulato, nervo centrali nervos adscendentes alternos utrinque 4 mittente quorum supremus a 3.5 cm. supra basin solutus, petiolo basi ima vaginante; pedunculo glabro petiolum aequante, spica florente quam limbus fere $\frac{1}{3}$ breviore tenui, rhachi hirsuta, bracteae pelta triangulari glabra pedicello latiusculo hirsuto, antheris subrotundis apice apiculatis, bacca puberula.

Ramuli spiciferi vix 1.5 mm. crassi; collenchyma libriforme in fasciculos directos dispositum, fasciculi intramedullares circiter 18, 1-seriati, canalis lysigenus nullus. Limbi in sicco firmo-membranacei minute pellucido-punctulati et virescentes, usque ad 12 cm. longi et 4 cm. lati. Petioli 5 mm. longi. Spicae florentes in sicco fuscescentes fere 1 mm. crassae. Stamina 3, filamenta rhachi inserta; bacca subtetragona, stigmata 3 sessilia.

COSTA RICA: Forêts de Las Vueltas, Tucurrique, alt. 700–800 m., *H. Pittier* 13187. In field, Juan Viñas, Reventazón Valley, alt. 1000 m., *O. F. Cook* and *C. B. Doyle* 294, 295.

Piper zentanum, n.sp.—Ramulis glabris; foliis modice petiolatis glabris; limbo ovato-elliptico basi aequilatera acuto apice acute acuminato, nervo centrali usque ad $\frac{1}{2}$ longitudinis suae nervos

adscendentes utrinque 4 mittente, nervo laterali adscendente utrinque a basi soluto, petiolo basi vaginante; pedunculo glabro quam petiolus paullo breviore, spica florente limbi dimidium aequante apice acuta, bracteae apice truncato-peltatae vertice triangulari glabro pedicello angusto dorso hirsuto, antheris rotundatis parvis, bacca obpyramidato-trigona glabra, stigmatibus linearibus brevibus.

Ramuli spiciferi 2 mm. crassi, collenchyma continuum zona interna libriforme, fasciculi intramedullares 1-seriati, canalis lysigenus centralis unicus. Limbi in sicco rigido-membranacei creberrime et minute pellucido-punctulati, usque ad 18 cm. longi et 10 cm. lati, nervuli primarii transverse paralleli. Petioli usque ad 20 mm., pedunculi 18 mm. longi. Spica florens usque ad 3 mm. crassa. Stamina 3. Stigmata 3 sessilia.

COSTA RICA: Hacienda de Zent, *A. Tonduz* 14649.

PIPER AEQUALE Vahl, ϵ **elliptico-lanceolatum**, n.var.—Limbo elliptico-lanceolato basi aequilatera acuto apice longius acuminato, usque ad 13 cm. longo et 7 cm. lato.

COSTA RICA: Collines de Piedades, près San Ramón, dans une haie de *Bromelia* et sur des troncs, *H. Pittier* 14185, 14186. Collines de Chirripó, alt. 1000 m., *A. Tonduz* 14655. Buissons et bords des chemins à Nicoya, *Tonduz* 13962. Forest at Matambu, Nicoya Peninsula, alt. 600 m., *O. F. Cook* and *C. B. Doyle*.

COLOMBIA: Santa Marta, *H. H. Smith* 385.

Piper minutantherum, n.sp.—Ramulis glabris minute verruculosis; foliis modice petiolatis glabris, limbo elliptico-lanceolato basi leviter inaequilatera utrinque acuto apice subacute et sat longe acuminato, nervo centrali nervos adscendentes altero latere 4 altero 5 mittente, quorum infimus a basi supremusque fere ex ½ longitudinis soluti, nervulo marginali a basi fere ad apicem ducto, petiolo basi ima vaginante; pedunculo glabro quam petiolus breviore, spica matura limbi dimidium aequante apice breviter acuta, rhachi glabra, bracteae pelta triangulari margine pedicelloque angusto hirtellis, antheris minutis quam filamenta tenuia multo brevioribus, bacca obovata glabra superne in stilum oblongum sat longe producta, stigmatibus minutissimis.

Ramuli spiciferi 2 mm. crassi, collenchyma in fasciculos discretos dispositum zona interna libriforme, fasciculi intramedullares 1-seriati, canalis

lysigenus unicus centralis. Limbi in sicco membranacei minute pellucido-punctulati, usque ad 21 cm. longi et 7.7 cm. lati. Petioli usque ad limbi latus longius 2.2 mm., inter limbi latera 2 mm. longi. Pedunculi 1.3 mm. longi. Spica 10 cm. longa, fere 4 mm. crassa. Stamina 3; bacca cum stilo 1.5 mm. longa.

GUATEMALA: In forest near Cubilquitz, *H. von Türckheim* II. 1441.

PIPER PELTAPHYLLUM C. DC., var. **lasvueltasanum,** n.var.—Limbo apice longe acuminato acumine obtusiusculo, bracteae pelta pedicelloque margine parce hirtellis.

COSTA RICA: Forêts de Las Vueltas, Tucurrique, alt. 635–900 m., *H. Pittier* 13189.

Piper grandilimbum, n.sp.—Ramulis glabris; foliis longe petiolatis magnis, limbo a circiter $\frac{1}{16}$ longitudinis peltato ovato-acuminato basi aequilatera haud profunde cordato apice longe et acute acuminato, supra glabro subtus ad nervos dense velutino-hirtello, 13-plinervio nervo centrali nervos adscendentes 3, quorum supremus fere ex 5 cm. supra basin solutus et sursum nervos validos utrinque mittente nervisque lateralibus utrinque 3 a petiolo divaricantibus, petiolo hirtello basi vaginante; pedunculo juniore hirtello dein glabro quam petiolus pluries breviore, spica submatura limbi dimidium superante, bracteae vertice subpeltato-triangulari margine hirtello pedicello angusto longius hirsuto, antheris globosis quam filamenta multo brevioribus, bacca glabra.

Frutex altus. Ramuli spiciferi 5 mm. crassi, collenchyma libriforme in fasciculos discretos dispositum, fasciculi intramedullares 2-seriati, canalis lysigenus unicus centralis. Limbi in sicco subrigidi minute et inconspicue pellucido-punctulati, superi usque ad 24 cm. longi et 13 cm. lati, inferi usque ad 29 cm. longi et 20 cm. lati. Petioli superi adulti 6.5 cm. longi, inferi alte supra basin vaginantes et usque ad 9 cm. longi. Pedunculus 2 cm. longus. Spica submatura in specimine viso incompleta 16 cm. longa et usque ad 4 mm. crassa canali lysigeno centrali munita. Stamina 3; bacca obpyramidato-trigona. Stigmata 3 sessilia cito decidua.—Species *P. peltaphyllo* proxima antheris rotundatis et minoribus, nervulis validis, collenchymate haud continuo ab illo discrepans.

GUATEMALA: Depart. Alta Verapaz prope Cubilquitz, alt. 350 m., in silva, *H. von Türckheim* II. 1490.

Piper pubinerve, n.sp.—Ramulis glabris; foliis modice petiolatis, limbo elliptico basi ima leviter inaequilatera acuto subacutove apice breviter acuminato, supra glabro subtus ad nervos minute

velutino, nervo centrali ex ultra $\frac{1}{2}$ longitudinis suae nervos adscendentes subrectos utrinque 7 mittente, petiolo usque ad limbum vaginante; pedunculo quam petiolus paullo breviore, spica quam limbus pluries breviore apice obtusa, bracteae inferne latae vertice truncato-peltato lunulato dense puberulo, antheris parvis rotundatoovatis, ovario libero apice in stilum mediocrem attenuato, bacca matura subtetragona.

Ramuli spiciferi circiter 2 mm. crassi, collenchyma sparsim libriforme in fasciculos discretos dispositum, fasciculi intramedullares 1-seriati. Limbi in sicco firmi pallidi minute pellucido-punctulati, usque ad 18.5 cm. longi et 11 cm. lati. Petioli 12 mm. longi. Spicae maturae 22 mm. longae et usque ad 1 cm. crassae, in sicco atrorubescentes. Stamina 4 rhachi inserta. Stigmata 2 lateralia linearia et sat longa.—Species quoad limbi formam *P. ripicolam* C. DC. valde referens, foliorum et bractearum pube ac antheris rotundatoovatis et brevioribus ab illo discrepans.

COSTA RICA: El General, alt. 600 m., *H. Pittier* 10607.

Piper virgultorum, n.sp.—Ramulis junioribus puberulis cito glabris et verruculosis; foliis modice petiolatis, limbo elliptico-lanceolato basi inaequilatera latere longiore subacuto breviore acuto, apice longe et acute acuminato, supra glabro subtus ad nervos adpresse hirtello, nervo centrali nervos adscendentes altero latere 5 altero 4 mittente quorum supremus fere a 6 cm. supra basin solutus, petiolo primum puberulo dein glabro et verruculoso basi ima vaginante; pedunculo glabro quam petiolus paullo breviore, spica florente quam limbi dimidium paullo breviore apice mucronata, rhachi glabra, antheris rotundato-reniformibus parvis quam filamenta oblonga paullo brevioribus, ovario inferne rhachi immerso superne libero glabro ovato-acuto, stigmatibus 2 linearibus transversalibus.

Ramuli spiciferi 1.5 mm. crassi, collenchyma in fasciculos discretos dispositum et zona interna libriforme, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco firme membranacei minute et inconspicue pellucido-punctulati, usque ad 18.5 cm. longi et 6.5 cm. lati. Petioli usque ad limbi latus longius 6 mm. inter limbi latera 2 mm. longi. Spicae florentes 6 cm. longae et 2 mm. crassae. Stamina 4.

COSTA RICA: Broussailles de Tsuritkub, Talamanca, alt. 100 m., *H. Pittier* 8650. Forêts sur les bords du Río Yurquin, *H. Pittier* 8570, cum spicis juvenilibus.

Piper tenuipes, n.sp.—Ramulis glabris tenuiter costulatis; foliis modice petiolatis parvis glabris, limbo ovato-lanceolato basi aequilatera acuto apice acute acuminato, petiolo basi ima vaginante; pedunculo glabro petiolum adultum subaequante tenui, spica florente quam limbus fere $\frac{1}{3}$ breviore tenui sublaxiflora, rhachi hirsuta, antheris reniformibus quam filamenta paullo brevioribus, ovario glabro ad rhachim elongato, stigmatibus ovatis obtusis.

Frutex 2–3 m. altus. Ramuli spiciferi 0.5 mm. crassi, collenchyma in fasciculos discretos dispositum haud libriforme, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco membranacei minute pellucido-punctulati, superi usque ad 7.5 cm. longi et 3.1 cm. lati. Petioli 7 mm., pedunculi circiter 6 mm. longi. Spicae florentes 3.6 cm. longae et 0.5 mm. crassae. Stamina 4. Stigmata 3 vel rarius 4.

COSTA RICA: Bois clairs et humides des collines de Piedades près San Ramón, alt. 1100 m., *A. Brenes* 14193. Lisière des bois humides au Cerro San Isidoro, près San Ramón, alt. 1300 m., *Brenes* 14196.

Piper cubilquitzianum, n.sp.—Ramulis glabris; foliis breviter petiolatis glabris, limbo elliptico-lanceolato basi aequilatera acuto apice longe et obtusiuscule protracto-acuminato, 5-nervio, petiolo basi ima vaginante; pedunculo glabro petiolum paullo superante, spica quam limbus paullo breviore, rhachi dense et breviter hirsuta, bractea obovata vertice inflexo nuda inferne subtus hirsuta, antheris reniformibus brevibus, baccis sublaxe dispositis ellipticis glabris, stigmatibus rotundatis.

Ramuli spiciferi 1 mm. crassi, collenchyma in fasciculos discretos lunulatos dispositum et haud libriforme, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco membranacei minute pellucido-punctulati, usque ad 12 cm. longi et 5 cm. lati. Petioli 5 mm., pedunculi 7 mm. longi. Stamina 4 rhachi inserta, bacca paullo sub 1 mm. longa.

GUATEMALA: Forests near Cubilquitz, Depart. Alta Verapaz, *H. von Türckheim* II. 1440.

Piper nicoyanum, n.sp.—Ramulis junioribus puberulis cito glabris; foliis modice petiolatis, limbo supra glabro subtus ad nervos hirtello, 7-nervio, petiolo hirtello basi vaginante; pedunculo juniore puberulo longitudine variabili quam petiolus breviore vel longiore, spica matura limbum paullo superante, rhachi hirsuta, bractea rotundata basi extus hirsuta, antheris reniformibus filamenta superantibus, ovario libero apice disculum orbiculare in

medio stigmatiferum gerente, stigmatibus ovatis, baccis fere globosis glabris.

Ramuli spiciferi 1 mm. crassi, collenchyma haud libriforme, in fasciculos discretos dispositum, fasciculi intramedullares 1-seriati, canalis lysigenus unicus centralis. Limbi in sicco membranacei crebre pellucido-punctulati, superi usque ad 8 cm. longi et 7 cm. lati. Petioli superi usque ad 1.5 cm. longi. Spicae maturae circiter 9 cm. longae. Stamina 4 basi ima baccae adnata, bacca circiter 1.5 mm. crassa. Stigmata 3–4, sessilia.—Vern. *alcotán*.

COSTA RICA: Bords des chemins à Nicoya, *H. Pittier* 13689, 13696.

Piper bryogetum, n.sp.—Ramulis glabris; foliis brevissime petiolatis glabris, limbo ovato-elliptico basi aequilatera rotundato apice acute acuminato, nervo centrali nervos adscendentes utrinque 2 mittente quorum supremus fere ex $\frac{1}{3}$ longitudinis centralis solutus, nervis lateralibus adscendentibus utrinque 2 a basi solutis, petiolo basi ima vaginante; pedunculo glabro petiolum aequante, spica matura quam limbi dimidium paullo breviore apice attenuata, bracteae pelta triangulari margine aureo-hirsuta pedicello angusto hirtello, bacca glabra vertice flavide glandulosa.

Frutex ad truncos muscosos scandens. Ramuli spiciferi fere 1 mm. crassi, collenchyma tenue continuum libriforme, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco membranacei pellucido-punctulati, circiter 13 cm. longi et 6 cm. lati. Petioli fere 10 mm. longi. Spica matura 3 mm. crassa. Stamina 4 ad basin baccae rhachi inserta, bacca obpyramidato-trigona. Stigmata 3 sessilia.

COSTA RICA: Forêts de Las Vueltas, Tucurrique, alt. 635 m., *H. Pittier* 12939. Le long du Río Hondo, plaines de Santa Clara, alt. 100 m., *O. F. Cook* and *C. B. Doyle* 563. Hacienda de Zent, alt. 30 m., *H. Pittier* 14652.

Piper longistipulum, n.sp.—Foliis breviter petiolatis subobovato-ellipticis, basi leviter inaequilatera utrinque rotundato-cordulatis, apice oblique et sat longe acuminatis, utrinque glabris, nervo centrali usque ad $\frac{1}{2}$ longitudinis suae nervos adscendentes alternos 4 et e basi 2–3 tenuiores utrinque mittente quorum infimi magis divaricantes; petiolo glabro basi vaginante, stipulis longis apice filiformibus; pedunculo glabro petiolum totum paullo superante; spica per anthesin limbi dimidium aequante apice sterili acuta, bracteae glabrae vertice triangulari inflexo, pedicello aequilato supra canaliculato.

Ramuli glabri, spiciferi 2 mm. crassi, collenchyma in fasciculos discretos dispositum et zona interna libriforme, fasciculi intramedullares 1-seriati,

canalis lysigenus nullus. Limbi in sicco membranacei pellucido-punctulati, 20 cm. longi, 7.5 cm. lati. Petioli usque ad limbi latus majus fere 8 mm., inter limbi latera 3 mm. longi. Stipulae membranaceae circiter 30 mm. longae. Spica per anthesin 8 cm. longa, fere 3 mm. crassa, in vivo atro-rubescens. Stamina 4, antherae subglobosae quam filamenta breviores. Bacca nondum matura tetragona lateraliter compressa. Stigmata 3 sessilia brevia.

COSTA RICA: Forêts du Río Naranja, alt. 200–250 m., *H. Pittier* 8001.

Piper melanocladum, n.sp.—Ramulis glabris in sicco nigris; foliis modice petiolatis glabris, limbo ovato-oblongo basi leviter inaequilatera altero latere rotundato altero acuto superne sat longe acuminato et apice obtusiusculo, nervo centrali nervos adscendentes utrinque 3 mittente quorum supremus paullo supra medium centralis solutus, nervis lateralibus adscendentibus altero latere 1 altero 2 a basi solutis, petiolo usque ad limbum vaginante; pedunculo glabro petiolum aequante, spica fere matura limbi dimidium paullo superante apice mucronulata, bracteae fere glabrae vertice triangulari et carnoso peltam simulante et margine infero puberulo, parte infero lata cucullata et glabra, antheris ovatis quam filamenta exserta multo brevioribus, bacca rotundato-subtetragona libera, stigmatibus oblongis apice acutis.

Ramuli spiciferi 2 mm. crassi, collenchyma in fasciculos discretos et sat crassos dispositum haud libriforme et cellulis brunneis intermixtum, fasciculi intramedullares 2-seriati. Limbi in sicco coriacei, superi usque ad 23 cm. longi et 7.5 cm. lati, subsequentes basi aequilatera rotundati usque ad 25 cm. longi et 9 cm. lati. Petioli usque ad limbi latus longius circiter 2 cm. et inter limbi latera 3 mm. longi. Spica in sicco nigra, bracteae coriaceae. Stamina 4, basi ima baccae adnata. Stigmata 3 sessilia.

COSTA RICA: Bords de l'Ariei, Talamanca, *H. Pittier* 9390. Forêts de Las Vueltas, Tucurrique, alt. 635–700 m., *Pittier* 13148.

Piper pallidifolium, n.sp.—Ramulis adpresse hirtellis; foliis brevissime petiolatis, limbo oblongo-elliptico-lanceolato basi leviter inaequilatera cordulato apice sat longe et acute acuminato, supra glabro subtus praesertim ad nervos nervulosque adpresse pilosulo, nervo centrali nervos adscendentes utrinque 3 quorum supremus fere ex $\frac{1}{2}$ longitudinis solutus, sursumque nervulos validos fere tota longitudine mittente, nervis lateralibus utrinque 2–3 a basi divaricantibus, petiolo hirsuto basi vaginante; pedunculo puberulo juvenili quam petiolus paullo breviore, spica subflorente quam limbus pluries breviore apice mucronata mucrone filiformi, bracteae

breviter spathulatae punctis rubellis notatae vertice inflexo mârgine infero puberulo, antheris ovatis.

Ramuli spiciferi 1 mm. crassi, collenchyma partim libriforme in fasciculos discretos dispositum, fasciculi intramedullares 1-seriati, canalis lysigenus nullus cellulis rubris in medulla crebris. Limbi in sicco membranacei cinerascentes rubello-pellucido-punctulati usque ad 12 cm. longi et fere usque ad 2.6 cm. lati. Petioli usque ad limbi latus longius 4 mm. inter limbi latera 2 mm. longi. Spicae in specimine adhuc juveniles 1.5 cm. longae et 1.5 mm. crassae. Stamina 4. Ovarium nondum formatum.

COSTA RICA: Bords de l'Ariei, Talamanca, *H. Pittier* 9392.

Piper pansamalanum, n.sp.—Ramulis hirtellis; foliis sat longe petiolatis, limbo ovato basi modice inaequilatera cordato lobis rotundatis apice breviter attenuato-acuto, supra glabro subtus ad nervos nervulosque hirsuto, nervo centrali nervos adscendentes utrinque 3 mittente quorum supremus fere ex $\frac{1}{2}$ longitudinis centralis solutus, nervis lateralibus utrinque 3 a basi divaricantibus, petiolo hirtello usque ad limbum vaginante; pedunculo glabro quam petiolus paullo breviore, spica limbum superante crassa apice attenuata, bracteae vertice inflexo-peltato triangulari margine hirsuto pedicello aequilato dorso hirsuto, antheris rotundatis quam filamenta multo brevioribus, bacca libera glabra tetragona, stigmatibus linearibus.

Ramuli spiciferi circiter 4 mm. crassi, collenchyma in fasciculos discretos crassos et confertos dispositum, non libriforme, fasciculi intramedullares 2-seriati. Limbi in sicco subcoriacei usque ad 26 cm. longi et 20 cm. lati, lobi basilares usque ad 5.5 cm. longi ad petiolum aequilongi. Petioli circiter 4 cm., pedunculi usque ad 5.5 cm. longi. Spica matura 40 cm. longa et usque ad 9 mm. crassa. Stamina 4 basi ima baccae adnata, antherae 0.5 mm. longae, stigmata 3 sessilia.

GUATEMALA: Pansamalá, Depart. Alta Verapaz, alt. 1140 m., *H. von Türckheim* 940. Silva supra Panzal, alt. 1200 m., *von Türckheim* II. 1703.

Piper magnilimbum, n.sp.—Ramulis glabris; foliis modice petiolatis, limbo elliptico basi valde inaequilatera cordato apice acute attenuato, supra glabro subtus ad nervos nervulosque puberulo, nervo centrali subtus squamis oblongis instructo nervos adscendentes utrinque 4–5 mittente quorum supremus paullo supra $\frac{1}{2}$ longitudinis solutus, nervis lateralibus utrinque 3–4 a basi divaricantibus, petiolo subtus squamis oblongis instructo et puberulo, usque ad limbi latus longius vaginante; pedunculo

minute puberulo petiolum fere aequante, spica limbum aequante, bracteae obovato-oblongae dorso puberulae vertice lanulato nudo et calloso, ovario libero glabro apice attenuato.

Collenchyma fere continuum zona interna libriforme, fasciculi intramedullares 3-seriati, canalis lysigenus nullus. Limbi in sicco firmi opaci, circiter 36 cm. longi et 26 cm. lati, lobi basilares rotundati discreti quorum major 10 cm. longus petiolum velans. Petioli usque ad limbi latus longius 7 cm., inter limbi latera 1–1.5 cm. longi. Spica post anthesin 6 mm. crassa apice acuta. Stamina 4 basi ovario adnata, stigmata 3 minuta sessilia et cito decidua.

COSTA RICA: Cañas Gordas, alt. 1100 m., *H. Pittier* 11032, 11073.

Piper euryphyllum, n.sp.—Ramulis glabris; foliis modice petiolatis, limbo ample ovato basi inaequilatero lateribus sat inaequilongis et parum inaequilatis, apice acuminato, supra glabro subtus ad nervos puberulo, nervo centrali nervos adscendentes utrinque 4 mittente quorum supremus paullo supra medium centralis solutus, nervis lateralibus utrinque 2 a basi solutis, petiolo subtus adpresse hirsuto usque ad limbum vaginante; pedunculo glabro quam petiolus paullo breviore, spica folium superante, bracteae cucullatae extus hirsutae intus glabrae vertice triangulari puberulo, ovario libero glabro, stigmatibus linearibus.

Ramuli spiciferi 5 mm. crassi, collenchyma in fasciculos discretos dispositum haud libriforme, cellulis fuscis intermixtum, fasciculi intramedullares 3-seriati. Limbi in sicco firmi fuscescentes pellucido-punctulati, usque ad 31 cm. longi et 21 cm. lati. Petioli circiter 5.5 cm. longi. Spica florens fere usque ad 1 cm. crassa. Stamina 4 basi ovarii adnata, bacca adulta verisimiliter tetragona, stigmata 3, sessilia.

COSTA RICA: Forêts de la Palma, alt. 1459 m., *H. Pittier* 12666.

Piper biseriatum, n.sp.—Ramulis dense villosis; foliis modice petiolatis, limbo subovato-elliptico basi valde inaequilatera cordato lobis conniventibus majore petiolum velante auriformi, apice sat longa acuminato, supra sparsim et longe piloso, subtus ad nervum centralem longe et ad paginam brevius et dense piloso, nervo centrali nervos adscendentes utrinque 3–4 mittente quorum supremus ex $\frac{1}{2}$ longitudinis centralis solutus, nervis lateralibus utrinque altero latere 2–3 altero 4–5 a basi divaricantibus, petiolo usque ad limbi latus brevius vaginante et dorso dense villoso, stipulis glabris; pedunculo glabro quam petiolus breviore, spica florente limbum superante, bracteae pelta ovato-triangulari inferne carnosa et

margine puberula superne membranacea et margine hirsuta, pedicello aequilato parce puberulo, antheris reniformibus.

Ramuli spiciferi fere 3 mm. crassi, collenchyma in fasciculos discretos dispositum, haud libriforme, fasciculi intramedullares 2-seriati, canalis lysigenus nullus. Limbi in sicco firmi opaci crebre pellucido-punctulati cum lobo auriformi usque ad 23.5 cm. longi et 11.5 cm. lati. Petioli usque ad limbi latus longius 3–3.5 cm., inter limbi latera 0.5 cm., pedunculi usque ad 2.5 cm. longi. Spica florens circiter 3 mm. crassa. Stamina 4.

COSTA RICA: Cañas Gordas, alt. 1100 m., *H. Pittier* 11036.

Piper pilibaccum, n.sp.—Ramulis altero latere sub foliis hirsutis; foliis breviter petiolatis, limbo ovato-oblongo basi leviter inaequilatera altero latere rotundato obtusove acuto apice acute et sat longe acuminato, supra glabro subtus praesertim ad nervos et nervulos hirtello, nervo centrali nervos patule adscendentes tenues utrinque 5 mittente quorum supremus fere ex $\frac{1}{2}$ longitudinis centralis solutus, petiolo subtus hirsuto fere usque ad medium vaginante; pedunculo glabro petiolum aequante, spica submatura quam limbus fere quadruplo breviore apice mucronulata, bractea obovata cucullata dorso et margine hirsuta, antheris reniformibus filamenta fere aequantibus, bacca libera trigona vertice dense hirsuta, stigmatibus subobovatis acutis.

Ramuli spiciferi 1 mm. crassi, adulti glabri, in 5 mm. crassis collenchyma continuum zona interna interrupta libriforme, fasciculi intramedullares 2-seriati, canalis lysigenus unicus centralis et tenuis. Limbi in sicco membranacei fusci minute pellucido-punctulati, usque ad 12.5 cm. longi et 4.5 cm. lati. Petioli usque ad limbi latus longius 4 mm., inter limbi latera 1.5 mm. longi. Spica submatura fere 4 cm. longa 1 mm. crassa. Stamina 4, bacca in vertice complanato rubiginose hirsuta. Stigmata 3.—Species *P. carpinteranum* C. DC. sat referens, bacca hirsuta ab illo discrepans.

COSTA RICA: In forest, Matambu, Nicoya Peninsula, alt. 600 m., *O. F. Cook* and *C. B. Doyle* 702.

PIPER SEPIUM C. DC., β **glabrum,** n.var.—Limbo utrinque glabro quam in specie longiore, usque ad 11 cm. longo et 2.5 cm. lato.

COSTA RICA: Cours supérieur du Diquís, *H. Pittier* 10570.

PIPER SEPIUM C. DC., γ **guacimonum,** n. var.—Limbo utrinque glabro et subtus nigro-punctulato, quam in specie paullo majore, foliorum superiorum usque ad 11.5 cm. longo et 4.5 cm. lato.

COSTA RICA: Hacienda de Guácimo, alt. 120 m., *A. Tonduz* 14656.

Piper nanum, n.sp.—Caule villoso inferne e nodis radicante; foliis brevissime petiolatis, limbo oblongo-ovato basi inaequilatera cordulato apice acute acuminato, utrinque et praesertim ad nervos villoso, nervo centrali nervos altero latera 4 altero 5 mittente quorum superi adscendentes, inferi subadscendentes, petiolo villoso usque ad limbi latus longius vaginante; pedunculo villoso petiolum aequante, spica juvenili petiolum paullo superante tenui apice tenuiter mucronata, bracteae cucullatae dorso hirtellae vertice inflexo nudo, antheris ovatis filamenta fere aequantibus.

Suffrutex circiter 30 cm. altus. Caulis inferne usque ad 3 mm., superne 1 mm. crassus, collenchyma in fasciculos discretos dispositum et zona interna libriforme, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco membranacei minute pellucido-punctulati, usque ad 14.5 cm. longi et 4.5 cm. lati. Petioli usque ad limbi latus longius 2 mm., inter limbi latera fere 1 mm. longi. Spica juvenilis 8 mm. longa et fere 1.5 mm. crassa. Stamina 4.

COSTA RICA: In atlantica declivitate, ad Guácimo, alt. 141 m., *A. Tonduz* 14659.

Piper Brenesii, n.sp.—Ramulis glabris; foliis breviter petiolatis glabris, limbo late elliptico basi leviter inaequilatera altero latere rotundato altero acuto, apice breviter et obtusiuscule acuminato, nervo centrali nervos adscendentes oppositos utrinque 6–7 mittente quorum supremus supra $\frac{1}{2}$ longitudinis centralis solutus, petiolo usque ad limbum vaginante; pedunculo glabro quam petiolus fere duplo breviore, spica quam limbus pluries breviore crassa apice mucronata, bracteae latae glabrae vertice inflexo triangulari, bacca libera glabra vertice rhomboidali.

Frutex 1–1.5 m. altus. Ramuli spiciferi 5 mm. crassi in sicco tetragoni, collenchyma in fasciculos discretos dispositum et zona interna libriforme, fasciculi intramedullares 2-seriati. Limbi in sicco firme membranacei crebre pellucido-punctulati, 20 cm. longi 11 cm. lati. Petioli 2 cm., pedunculi 1 cm. longi. Spicae maturae 5 cm. longae et 6 mm. crassae. Stamina 4 basi ima baccae adnata. Stigmata 3, sessilia cito caduca.

COSTA RICA: Petits bois des collines de Santiago à San Ramón, *Brenes* 14190.

Piper mombachanum, n.sp.—Ramulis glabris; foliis modice petiolatis glabris, limbo oblongo-elliptico-lanceolato basi inaequilatera altero latere subrotundato altero acuto, apice acute et sat longe acuminato, nervo centrali nervos adscendentes utrinque

4–5 mittente quorum supremus fere ex $\frac{1}{2}$ longitudinis centralis solutus, petiolo basi ima vaginante; pedunculo glabro petiolum paullo superante, spica matura limbi dimidium fere aequante, apice mucronulata vel obtusa, bracteae pelta triangulari fere glabra pilis aliquot brevibus in marginibus lateralibus munita, pedicello subglabro angusto, antheris reniformibus quam filamenta paullo brevioribus, bacca libera tetragona lateraliter compressa apice minute puberula, stigmatibus linearibus.

Frutex divaricato-ramosus 3–3.5 m. altus. Ramuli spiciferi 1 mm. crassi, collenchyma subcontinuum zona interna libriforme, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco membranacei crebre pellucido-punctulati, 14.5–15.5 cm. longi, 4–5 cm. lati. Petioli usque ad limbi latus longius 7 mm., inter limbi latera 3 mm., pedunculi 15 mm. longi. Spica matura 8 cm. longa, 2 mm. crassa. Stamina 4 baccae alte adnata. Stigmata 3 sessilia.

NICARAGUA: Volcán Mombacho, Depart. Granada, in deep woods, *C. F. Baker* 2462.

COSTA RICA: Bois de San Pedro près San Ramón, alt. 1300–1400 m., *A. Tonduz* 17765, 17784.

Piper flaviramum, n.sp.—Ramulis parce hirtellis; foliis brevissime petiolatis glabris, limbo subovato-oblongo basi leviter inaequilatera obtuso, apice longe acuminato, nervo centrali nervos adscendentes utrinque 5 mittente quorum supremus fere a $\frac{1}{4}$ longitudinis nervi centralis solutus, petiolo basi ima vaginante; pedunculo glabro petiolum multo superante; spica juvenili quam limbus pluries breviore in sicco rubescente, bracteae pelta triangulari margine hirsuta pedicello sat angusto glabro, ovario libero ovato-acuto glabro.

Scandens, ramuli in sicco flavi, teretes, spiciferi fere 1 mm. crassi, collenchyma continuum libriforme, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco firme membranacei, flavicantes, minute pellucido-punctulati, circiter 16 cm. longi et 4.5 cm. lati. Petioli fere 3 mm., pedunculi fere 7 mm. longi. Spica juvenilis 2.5 cm. longa et 1.5 mm. crassa. Stamina 4. Stigmata 3.—Species *P. xanthostachio* proxima ab illo foliis subsessilibus, ramulis hirtellis et spicarum juvenilium colore sat discrepans.

COSTA RICA: Forêts de La Palma, alt. 1450 m., *H. Pittier* 12510.

Piper gibbifolium, n.sp.—Ramulis glabris; foliis modice petiolatis, limbo elliptico-obovato basi inaequilatero, lateribus ad petiolum aequilongis valde inaequilatis altero rotundato altero

acuto, apice acuminato, supra glabro subtus ad nervos nervulosque puberulo, nervo centrali nervos altero latere 5 altero 8 mittente quorum supremus fere ex $\frac{1}{2}$ longitudinis centralis solutus, petiolo glabro basi ima vaginante; pedunculo glabro; spica subflorente quam limbus pluries breviore apice subacuta, bracteae pelta triangulari margine pedicelloque angusto hirsutis, antheris ovatis.

Ramuli in sicco nigrescentes, spiciferi 2 mm. crassi, in 4 mm. crassis collenchyma in fasciculos a latere valde productos dispositum seu subcontinuum et zona interna libriforme, fasciculi intramedullares 2-seriati, canalis lysigenus unicus centralis. Limbi in sicco firme membranacei, parce pellucido-punctulati, fere 22 cm. longi et 11 cm. lati apice in specimine viso incompleti. Petioli usque ad limbi latus longius 13 mm., inter limbi latera 3 mm., pedunculi 8 mm. longi. Spica subflorens 6 cm. longa et basi 3 mm. crassa. Stamina 4.

COSTA RICA: Hacienda de Zent, alt. 31 m., *A. Tonduz* 14649 in Herb. Inst. Fís. Geogr. Costar. et Herb. Berol., non eadem planta ut eodem numero in Herb. Boiss.

Piper brachistopodum, n.sp.—Ramulis glabris; foliis brevissime petiolatis, limbo elliptico basi leviter inaequilatera altero latere rotundato altero acuto apice acute acuminato, supra glabro subtus ad nervos minute puberulo, nervo centrali nervos alternos adscendentes altero latere 4 altero 5 mittente quorum supremus ex $\frac{1}{2}$ longitudinis centralis solutus, petiolo basi ima vaginante; pedunculo glabro quam petiolus breviore, spica matura limbi dimidium vix aequante apice obtusa, bracteae pelta parva triangulari margine infero hirtella pedicello glabro inferne cuneato, antheris rotundatis quam filamenta brevioribus, bacca subtetragona glabra, stigmatibus minutis.

Ramuli spiciferi circiter 3 mm. crassi, collenchyma in fasciculos discretos dispositum zona interna libriforme, fasciculi intramedullares 1-seriati. Limbi in sicco membranacei pellucido-punctulati, circiter 21 cm. longi et 9 cm. lati. Petioli usque ad limbi latus longius 5 mm., inter limbi latera 3 mm., pedunculi adulti 4 mm. longi. Spica matura 9 cm. longa, 4 mm. crassa. Stamina 4 supra medium baccae inserta. Stigmata 3 sessilia.

COSTA RICA: Forêts de Las Vueltas, Tucurrique, alt. 635–700 m., *A. Tonduz* 13143.

Piper uvitanum, n.sp.—Ramulis hirtellis; foliis brevissime petiolatis, limbo ovato-elliptico basi leviter inaequilatera utrinque obtuso, apice acute acuminato, supra glabro subtus velutino-pubescente, nervo centrali fere ex $\frac{1}{2}$ longitudinis suae nervos patule

adscendentes utrinque 4–5 sursumque nervulos saepe validos mittente, petiolo hirtello basi vaginante; pedunculo hirtello petiolum subaequante, spica submatura quam limbus fere quadruplo breviore apice obtusa, bracteae vertice truncato lunulato-triangulari margine dense et fulvescente hirsuto pedicello sat lato extus hirsuto, antheris rotundatis parvis, bacca tetragona vertice dense et fulvescente hirsuta.

Ramuli spiciferi circiter 2 mm. crassi, collenchyma libriforme in fasciculos discretos a latere productos dispositum seu subcontinuum, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco membranacei creberrime pellucido-punctulati, usque ad 20 cm. longi et 10 cm. lati. Petioli usque ad limbi latus longius 10 mm., inter limbi latera 3 mm. longi. Spica submatura in sicco fulvescens fere 4 mm. crassa. Stamina 4 alte cum bacca connata. Stigmata 3 sessilia.

COSTA RICA: Îlot de la Uvita, Puerto Limón, *H. Pittier* 12690.

PIPER SUBSESSILIFOLIUM C. DC., *β* **palmanum,** n.var.—Pilis ramulorum haud retrorsis.

COSTA RICA: Forêts de La Palma, alt. 1459 m., *H. Pittier* 12662.

Piper vestitifolium, n.sp.—Ramulis villosis; foliis breviter petiolatis, limbo oblongo-ovato-acuminato basi inaequilatera altero latere rotundato altero acuto, apice acute et sat longe acuminato, utrinque dense hirsuto, nervo centrali nervos adscendentes utrinque 5 mittente quorum supremus paullo infra medium centralis solutus, petiolo dense hirsuto basi ima vaginante; pedunculo dense hirsuto petiolum fere aequante, spica limbi dimidium subaequante apice acuta, bracteae pelta triangulari margine pedicelloque angusto hirsutis, antheris rotundatis parvis quam filamenta oblonga brevioribus, bacca subtetragona apice parce pilosula, stigmatibus linearibus brevissimis.

Ramuli spiciferi 1.5 mm. crassi, in 3 mm. crassis collenchyma in fasciculos discretos dispositum, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco membranacei minute pellucido-punctulati, 10.5 cm. longi, 4 cm. lati. Petioli usque ad limbi latus longius 2 mm., inter limbi latera 3 mm., pedunculi 6 mm. longi. Spica submatura 6 cm. longa usque ad 3 mm. crassa. Stamina 4 basi baccae adnata, stigmata 3 sessilia.

GUATEMALA: Near the Finca Sepacuité, Alta Verapaz, *O. F. Cook* and *R. F. Griggs* 651.

Piper perhispidum, n.sp.—Ramulis dense hirsutis pilis sat longis; foliis breviter petiolatis, limbo oblongo-ovato-acuminato

basi inaequilatera utrinque rotundato, apice acute et sat longe acuminato, utrinque sat longe hirsuto, adulto supra scabro et bullato, nervo centrali nervos adscendentes utrinque 5 mittente quorum supremus paullo infra medium solutus, petiolo dense hirsuto basi ima vaginante; pedunculo hirsuto petiolum fere aequante, spica matura limbi dimidium paullo superante apice obtuse mucronata mucrone hirsuto, bracteae pelta triangulari margine pedicelloque angusto hirsutis, antheris reniformibus, bacca tetrágona lateraliter compressa apice dense hirtella, stigmatibus linearibus.

Ramuli spiciferi 2.5 mm. crassi pilis 1.5 mm. longis; collenchyma continuum zona interna libriforme, fasciculi intramedullares partim 2-seriati, canalis lysigenus nullus. Limbi in sicco rigidi creberrime pellucido-punctulati, usque ad 17 cm. longi et ad 7 cm. lati. Petioli usque ad limbi latus longius 5 mm., inter limbi latera 3 mm. longi. Spicae maturae 7.5 cm. longae et usque ad 2.5 mm. crassae in sicco fuscescentes. Stamina 4 baccae supra basin adnata. Stigmata 3 sessilia.

COSTA RICA: Bords du Río Barranca, près San Juan de San Ramón, alt. 1500–1600 m., *A. Tonduz* 17771.

Piper submultiplinerve, n.sp.—Ramulis glabris; foliis longiuscule petiolatis glabris, limbo oblongo-ovato basi leviter inaequilatera utrinque acuto, apice acute acuminato, nervo centrali nervos adscendentes altero latere 4 altero 5 mittente, quorum supremus ex $\frac{1}{3}$ longitudinis centralis solutus et infimus basi proximus, petiolo ultra medium vaginante; pedunculo puberulo quam petiolus multo breviore, spica florente quam limbus fere triplo breviore apice acuta, bracteae pelta lunulata margine pedicelloque aequilato flavo-hirsutis, antheris exsertis ovatis quam filamenta multo brevioribus, ovario glabro apice attenuato.

Ramuli spiciferi 2 mm. crassi verruculis concoloribus asperati, collenchyma continuum zona interna libriforme, fasciculi intramedullares 1-seriati, canales lysigeni 2 in medulla. Limbi in sicco membranacei crebre pellucido-punctulati, fere usque ad 15.5 cm. longi et 7 cm. lati. Petioli usque ad limbi latus longius 30 mm., inter limbi latera 3 mm. longi. Stamina 4, stigmata 3.

COSTA RICA: Vallée de Los Orcángeles, massif de Iscazú, alt. 1700 m., *H. Pittier* 13626.

Piper leptoneuron, n.sp.—Ramulis glabris; foliis modice petiolatis glabris, limbo elliptico-lanceolato basi aequilatera acuto,

apice acute acuminato, nervo centrali nervos adscendentes tenues utrinque 4 mittente quorum supremus fere e medio centralis solutus, petiolo basi ima vaginante; pedunculo glabro petiolum aequante, spica juvenili quam limbus fere quadruplo breviore, bracteae vertice truncato margine pedicelloque hirsutis.

Ramuli laeves, spiciferi 1 mm. crassi, collenchyma continuum zona interna partim libriforme, fasciculi intramedullares 2-seriati. Limbi in sicco membranacei opaci parce pellucido-punctulati, 8.5–9 cm. longi, 3–4 cm. lati, acumen 10 mm. longum. Petioli circiter 10 mm longi. Spica in specimine adhuc juvenilis 1.5 mm. crassa. Stamina 4.

COSTA RICA: Santa Clara, Las Delicias, alt. 500 m., *H. Pittier* 10675.

Piper nodosum, n.sp.—Ramulis glabris; foliis brevissime petiolatis, limbo elliptico-lanceolato basi levissime inaequilatera utrinque acuto, apice acuminato, supra glabro subtus ad nervos minutissime puberulo, nervo centrali nervos adscendentes sat tenues utrinque 5 mittente quorum supremus e medio centralis solutus, petiolo glabro basi ima vaginante, pedunculo glabro petiolum aequante, spica limbi dimidium superante; bracteae apice truncatae vertice triangulari margine antico minute puberulo pedicello dorso parce hirtello, antheris minutis quam filamenta multo brevioribus, bacca glabra tetragona.

Ramuli laeves in sicco pallidi, spiciferi 1.5 mm. crassi, collenchyma in fasciculos discretos dispositum et zona interna libriforme, fasciculi intramedullares 1-seriati. Limbi in sicco membranacei crebre pellucido-punctulati, usque ad 16 cm. longi et 6 cm. lati. Petioli vix 5 mm. longi. Spica matura circiter 11 cm. longa et usque ad 4 mm. crassa apice obtusa. Stamina 4 basi ima baccae adnata. Stigmata 3 sessilia.

COSTA RICA: Cañas Gordas, alt. 1100 m., *H. Pittier* 11072.

Piper diquisanum, n.sp.—Ramulis glabris verruculis asperatis; foliis breviter petiolatis glabris, limbo oblongo-ovato-lanceolato basi parum inaequilatera utrinque acuto, apice longe et acute acuminato, nervo centrali nervos adscendentes utrinque 5 mittente quorum supremus fere a medio centralis solutus petiolo basi ima vaginante; pedunculo glabro petiolum duplo superante, spica florente limbi dimidium fere aequante apice obtusa, bracteae pelta triangulari parva margine minute puberula pedicello angusto antheris ovato-rotundis filamenta fere aequantibus, ovario glabro, stigmatibus linearibus.

Ramuli spiciferi vix 2 mm. crassi, collenchyma subcontinuum zona interna libriforme, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco membranacei crebre pellucido-punctulati fere 15 cm. longi et 4 cm. lati. Petioli usque ad limbi latus longius 7 mm., inter limbi latera circiter 8.5 mm., pedunculi fere 22 mm. longi. Spicae florentes circuiter 8.5 cm. longae et 3 mm. crassae. Stamina 4. Stigmata 3 sessilia.

COSTA RICA: Cours supérieur du Diquís, *H. Pittier* 10567.

Piper chirripoense, n.sp.—Ramulis glabris; foliis breviter petiolatis, limbo elliptico-lanceolato basi leviter inaequilatera utrinque acuto, apice acute et longiuscule acuminato, supra remote piloso subtus ad nervos dense piloso et ad paginam puberulo, nervo centrali nervos adscendentes utrinque 7 mittente quorum supremus supra medium centralis solutus, petiolo subtus dense piloso usque ad limbum vaginante; pedunculo subglabro quam petiolus breviore, spica florente quam limbus pluries breviore apice mucronulata, bracteae cucullatae glabrae vertice triangulari; antheris subglobosis filamenta aequantibus, stigmatibus linearibus.

Ramuli lenticellis parvis conspersi, collenchyma in fasciculos discretos dispositum zona interna sparsim libriforme. Limbi in sicco membranacei usque ad 19 cm. longi et 10 cm. lati. Petioli usque ad limbi latus longius 10 mm., inter limbi latera 2 mm., pedunculi 5 mm. longi. Spica florens 2.5 cm. longa, 1.5 mm. crassa. Stamina 4. Stigmata 3 sessilia.

COSTA RICA: Bois frais au pied des collines de Chirripó, alt. 100 m., *H. Pittier* 16061.

PIPER CITRIFOLIUM Lam., **γ Cookii,** n.var.—Ramulis glabris, limbo ovato-lanceolato basi inaequilatera altero latere rotundato altero acuto, usque ad 14.8 cm. longo et 5.9 cm. lato.

GUATEMALA: Near the Finca Sepacuité, Alta Verapaz, *O. F. Cook* and *R. F. Griggs* 697.

Piper subcitrifolium, n.sp.—*Piper uspantanense* C. DC. BOT. GAZ. **19**:6. 1894, in part.—Ramulis glabris; foliis brevissime petiolatis, limbo elliptico-lanceolato basi inaequilatera utrinque acuto, apice acute acuminato, supra glabro subtus ad nervos nerv ulosque minute velutino, nervo centrali nervos adscendentes utrinque 5 mittente quorum supremus fere a medio centralis solutus, petiolo puberulo basi ima vaginante; pedunculo subglabro quam petiolus breviore, spica quam limbus paullo breviore apice obtusa bracteae vertice truncato triangulari margine dense et flavide hirsuta,

pedicello aequilato margine hirtello, bacca inferne haud profunde in rhachi immersa et cum ea concreta, vertice pulposa et hirsuta.

Ramuli spiciferi 2.5 mm. crassi, collenchyma continuum zona interna sparsim libriforme, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco membranacei creberrime et minute pellucido-punctulati, usque ad 13 cm. longi et 6 cm. lati. Petioli usque ad limbi latus longius fere 10 mm., inter limbi latera 2 mm., pedunculi 6 mm. longi. Spica matura 10 cm. longa usque ad 3.5 mm. crassa. Stamina 4; baccae flavicantes. Stigmata 3 sessilia.

GUATEMALA: Cerro Gordo, Depart. Santa Rosa, *J. D. Smith* 3827.

Piper triseriale, n.sp.—Ramulis hirtellis; foliis sat longe petiolatis, limbo ample ovato basi modice inaequilatera utrinque truncato-rotundato, apice brevissime acuminato, supra glabro subtus ad nervos nervulosque hirtello, nervo centrali nervos adscendentes utrinque 5 mittente quorum supremus fere e medio centralis solutus, nervis lateralibus utrinque 3 a basi solutis, petiolo subtus hirsuto usque ad limbi latus brevius vaginante stipulis dorso hirtellis; pedunculo glabro petiolum aequante, spica limbum aequante vel paullo superante, bracteae cucullatae vertice inflexo rotundato-triangulari pedicelloque calceoliformi margine hirsutis, antheris reniformibus parvis, bacca glabra submatura rotundato-subtetragona, stilo brevi, stigmatibus linearibus.

Ramuli spiciferi circiter 8 mm. crassi in sicco nigri, collenchyma in fasciculos discretos dispositum et haud libriforme, fasciculi intramedullares 3-seriati, canalis lysigenus nullus. Limbi in sicco coriacei pellucido-punctulati, usque ad 30 cm. longi et fere 20 cm. lati, in specimine verisimiliter abnormaliter inferne margine obtuse sinuato-dentati. Petioli usque ad limbi latus longius 6 cm., inter limbi latere 6 mm. longi. Spica fere matura 39 cm. longa, 6 mm. crassa. Stamina 4 basi baccae adnata. Stilus bacca multo brevior. Stigmata 3.

COSTA RICA: Forêts de La Palma, alt. 1459 m., *H. Pittier* 12663.

Piper dasypogon, n.sp.—Ramulis longe et fusce pilosis; foliis magnis modice petiolatis, limbo oblongo-elliptico basi valde inaequilatera profunde cordato, lobo majore auriformi petiolum velante, apice sat longe acuminato, supra laevi praesertim ad nervos subtus densius et ubique piloso, nervo centrali nervos adscendentes utrinque 3–4 mittente, quorum supremus fere a medio centralis solutus, nervis lateralibus 4 e basi in lobum majorem divaricantibus, petiolo subtus dense et longe piloso usque ad limbi lobum majorem

vaginante; pedunculo longe piloso gracili quam petiolus fere duplo longiore, spica quam limbus fere $\frac{1}{3}$ breviore, sat crassa, bracteae adultae glabrae inferne canaliculatae vertice cucullato subrotundo margine praesertim postice longe piloso, antheris rotundatis quam filamenta pluries brevioribus, ovario glabro in stilum attenuato, stigmatibus linearibus.

Ramuli spiciferi circiter 4 mm. crassi, collenchyma haud libriforme in fasciculos discretos dispositum, fasciculi intramedullares 1-seriati, canalis lysigenus unicus centralis. Limbi in sicco firmi opaci et pellucido-punctulati usque ad 32 cm. longi et 14 cm. lati. Petioli usque ad limbi latus longius 3.5 cm., inter limbi latera 7 mm. longi, lobus basilaris major fere 7 cm. longus. Pedunculi 7.5 cm. longi. Spicae usque ad 6 mm. crassae. Stamina 4 ad basin ovarii rhachi inserta, bacca verisimiliter stilifera.

COSTA RICA: Buenos Aires, alt. 2000 m., *H. Pittier* 10641.

Piper sinugaudens, n.sp.—Ramulis altero latere villosis; foliis brevissime petiolatis, limbo elliptico-oblongo basi inaequilatera cordulato, apice longe et acute acuminato, supra glabro subtus ad nervos nervulosque hirtello, nervo centrali nervos adscendentes utrinque 5 mittente quorum supremus a medio centralis solutus, petiolo dense hirsuto basi vaginante; pedunculo hirtello petiolum multo superante, spica quam limbus pluries breviore apice obtusiuscula, bracteae calceoliformis dorso hirsutae vertice triangulari margine hirsuto, antheris rotundatis filamenta fere aequantibus, ovario glabro apice in stilum attenuato, stigmatibus linearibus.

Ramuli spiciferi 2 mm. crassi, in 2.5 mm. crassis collenchyma in fasciculos discretos a latere valde productos dispositum et haud libriforme, fasciculi intramedullares 1-seriati, canalis lysigenus nullus. Limbi in sicco membranacei obscure virides parce pellucido-punctulati, superi usque ad 17.5 cm. longi et 5.2 cm. lati. Petioli usque ad limbi latus longius et inter limbi latera 2 mm., pedunculi 12 mm. longi. Spicae florentes 5.2 cm. longae, 2 mm. crassae. Stamina 4. Stigmata 2.

COSTA RICA: Buena Vista, road to San Carlos Valley, alt. 600 m., in deep ravine, *O. F. Cook* and *C. B. Doyle* 150.

Piper calcaratum, n.sp.—Ramulis glabris; foliis breviter petiolatis, limbo oblongo-obovato basi leviter inaequilatera altero latere subrotundato altero acuto, apice acute acuminato, supra glabro subtus ad nervos puberulo, nervo centrali nervos adscendentes utrinque 7 mittente quorum supremus fere a 13.5 cm. supra basin solutus, petiolo glabro usque ad limbum vaginante;

pedunculo glabro petiolum fere aequante, spica florente quam limbus fere triplo breviore apice mucronato, bractea lata florem antice amplectente glabra inferne dorso longe calcarata, calcare subulato, ovario stiloque glabris, stigmatibus brevibus linearibus.

Ramuli spiciferi circiter 4 mm. crassi, collenchyma in fasciculos discretos dispositum et zona interna sparsim libriforme. Limbi in sicco membranacei minute pellucido-punctulati, circiter 26 cm. longi et 12.5 cm. lati. Petioli usque ad limbi latus longius fere 20 mm., inter limbi latera fere 3 mm. longi. Spica florens circiter 7 cm. longa et inferne cum stilis circiter 7 mm. crassa, bracteae calcar 1 mm. longum. Stamina 4 ad imam basin baccae rhachi inserta, stilus 2 mm. longus. Stigmata 3.

COSTA RICA: Forêts de Las Vueltas, Tucurrique, alt. 635 m., *H. Pittier* 13185.

Piper acuminatissimum, n.sp.—Ramulis glabris; foliis modice petiolatis glabris, limbo oblongo-elliptico-lanceolato basi aequilatera acuto, apice sat longe et acute acuminato, nervo centrali nervos adscendentes utrinque circiter 10 mittente quorum supremus supra medium centralis solutus, petiolo basi ima vaginante; pedunculo glabro quam petiolus breviore, spica submatura quam limbus pluries breviore, bractea galeato-ovata glabra rhachi glabra, baccis sublaxe dispositis subtetragonis glabris, stigmatibus brevibus linearibus.

Ramuli spiciferi fere 2 mm. crassi, collenchyma in fasciculos discretos dispositum et omnino libriforme, fasciculi intramedullares 1-seriati, cellulae flavidae in medulla et in cortice sparsae. Limbi in sicco membranacei et virescentes, crebre pellucido-punctulati usque ad 17 cm. longi et 6.5 cm. lati. Petioli usque ad 18 mm., pedunculi 6 mm. longi. Spicae submaturae fere 4 cm. longae. Stamina 4. Baccae in sicco rubrae, fere 3 mm. longae. Stigmata 4 sessilia.

COSTA RICA: Plaines du San Carlos, alt. 100 m., *H. Pittier* 16321.

PIPER PULCHRUM C. DC. γ **copeyanum,** n.var.—Limbo basi fere aequilatera profunde cordato vel breviter peltato apice obtuse acuminato, usque ad 18 cm. longo et fere 11 cm. lato; spica submatura quam limbus paullo longiore.

COSTA RICA: Forêts de Santa Rosa de Copey, alt. 1800 m., *H. Pittier* 12198.

STUDIES IN EVAPORATION AND TRANSPIRATION[1]

GEO. F. FREEMAN

(WITH FIVE FIGURES)

As the result of studies extending over several years, considerable experimental data have been accumulated concerning the transpiration of alfalfa and other plants under various accurately measured conditions of temperature, relative humidity, and wind movement. It was found, however, that the interpretation of these results was hopeless unless something more was known about the quantitative influence of these factors upon purely physical evaporation than was available to the writer. Thus it was impossible to say whether a given result was simply the product of the physical factors then obtaining, or to what extent it was modified by the physiological reactions of the plant itself. One should be able to calculate from rational formulae the behavior of a physical evaporating surface for the given conditions. Any departure from this result could then be attributed to the response of the living organism.

It may be assumed that the general type of such a formula will remain the same for all situations, but that the constants will vary with all of the varying conditions of environment, temperature, wind movement, relative humidity, and size and nature of the evaporating surface. The purely physical experiments here reported were designed, first to work out the generalized type of the formula, and then to obtain the constants for certain special cases of environment and other factors. Environment is used here in a restricted sense, meaning only the size and nature of the space within which the evaporating surface is inclosed. In the work reported this was one liter of space included within a glass cylinder closed by two large rubber stoppers at either end. When

[1] These experiments were conducted at the University of Arizona Agricultural Experiment Station during the writer's connection with that institution, which extended over the years 1909–1918. They were, for the most part, conducted during the latter part of this period.

a surface is evaporating in the open, the environment is unbounded or indefinite. It differs from the closed environment, not in type, but in degree, since where there is any wind movement, after a greater or less length of time, the evaporating surface will come into equilibrium with it, and thereafter give off water at a uniform rate. The larger the environment and the slower the wind movement, the longer will be the time required to reach this equilibrium. With an unbounded environment and no wind movement, theoretically it would never be reached, but for all practical purposes a small evaporating surface exposed in the open in still air soon reaches a uniform rate of evaporation, which will remain constant so long as the temperature and dewpoint of the air remain the same. The influence of the size of the environment, therefore, is capable of mathematical expression, and may enter as a factor in a rational evaporation formula. When the size of the environment always remains the same, as in all the experiments conducted by the writer, this factor may be left out of the formulae as a variable, that is, its value may be included in one of the other constants found. When the size or nature of the environment varies in any way, it must be calculated as a separate constant, or else the other constants (which had included it) must be recalculated for each new environmental condition. It is this consideration which has made it impossible to write, for any given type of surface, an evaporation formula which would apply to all situations, even though all be in the open. The reason for this is that every environment is more or less restricted by buildings, trees, banks, hills, or mountains, so that no two are exactly alike. It would be impossible to write a formula which would account for all of these variations. So long, however, as we confine ourselves to a given environment and a given type of evaporating surface, we can easily account for variations in amount of surface, temperature, wind movement, and dewpoint of the air.

In the physical experiments undertaken, the evaporating surface used was a porous cup atmometer. A constant stream of air was drawn through the cylinder (over the atmometer) by a rotary air pump driven by a small motor. The air was measured by a water gasometer which was accurate to 1/1000 of a cubic foot.

The dewpoint of the incoming and outgoing air was determined by the interjection of the base of a polished nickel test-tube into the air stream. Ether within the tube was cooled by blowing a current of air into it with an atomizer bulb. When the ether and the tube were cooled to the dewpoint of the air stream, the formation of a film of dew on the outside of the test-tube could be observed. A delicate thermometer having its bulb immersed in the ether was then quickly read to 0.1 of a degree, which appeared to be sufficiently accurate for all practical purposes. In the following discussion only the dewpoints of the incoming and outgoing air are given. Anyone desiring to know the absolute evaporation can quickly calculate it from these data; but for the purposes of this discussion this is not necessary. Let y = rise in dewpoint of outgoing air over that of the incoming air; t = temperature of air; t_1 = dewpoint of incoming air; z = a constant, the value of which will depend upon the rate of air movement (w) and the size and nature of the evaporating surface. The value of y may then be expressed by the formula $y = z(t - t_1)$. The agreement of this formula with the experimental results is shown in fig. 1. This gives the results of an experiment in which the temperature was artificially raised and controlled, giving a range from 16.4 to 25.2° C. Since the dewpoint of the air used remained constant at 3.0°, there was a range in the value of $t - t_1$ of 8.8°. The lines ot and ot_1 give the value of t and t_1 respectively. The line oy gives the calculated value of y when $z = 53$ per cent. The dots show the agreement of the experimental with the calculated value of y. So long as other factors remain the same, the value of y appears to

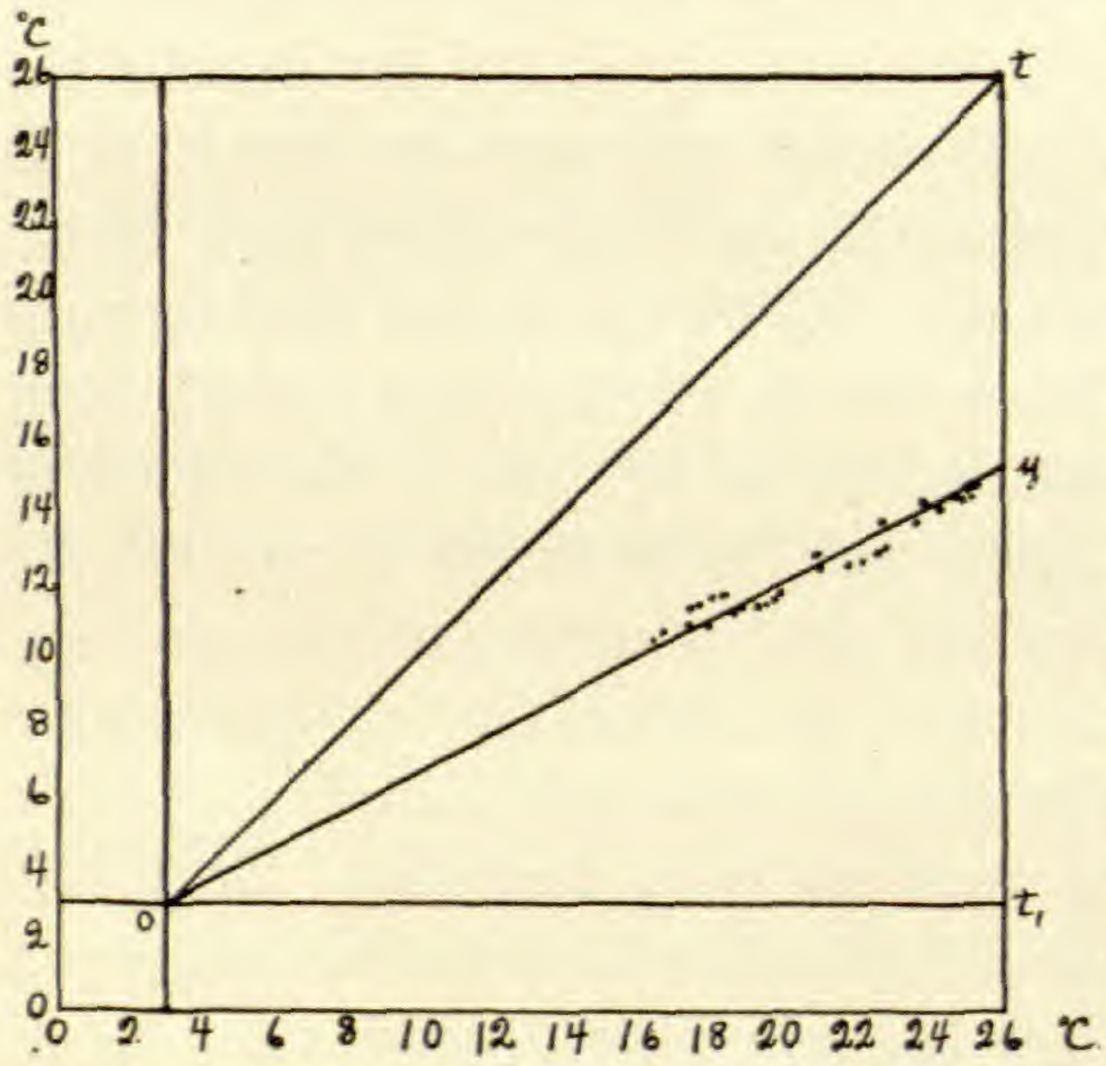

FIG. 1

be a constant linear proportion of the rise of t. A number of other experiments were made which exhibited a similar agreement between the experimental and calculated results. All of these

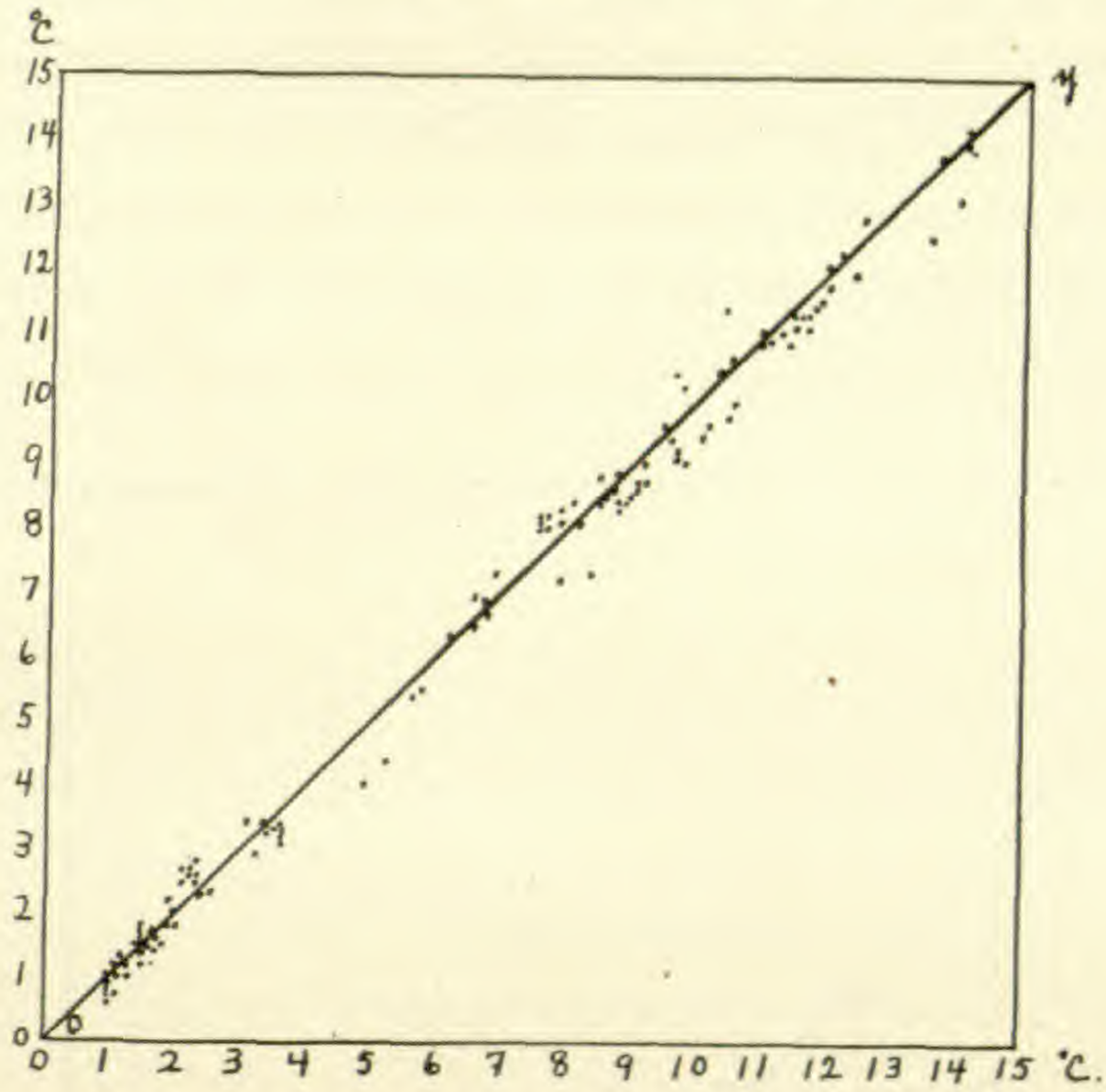

FIG. 2

are summarized in fig. 2, in which the ascending line oy represents the calculated value of y, while the dots show the experimental results obtained.

Effect of wind movement

It has been stated that the value of z depends upon the rate of air movement (w) and the extent and nature of the evaporating surface. When the latter remains unchanged and only wind movement is varied, z takes the form of $z=\frac{1}{1+c(w)^n}$, in which c and n are constants. The formula then becomes $y=\frac{t-t_1}{1+c(w)^n}$. In using this formula it is convenient to plot the results as is shown in fig. 3; ot is the temperature of the air, and o_1t_1 is its dewpoint; both of which are here constant at 21° and −1.8° respectively. The rate of wind movement may be plotted along o_1t_1, from which the found values of y may be plotted as ordinates. The curved line oy joining these ordinates then gives the value of y, which is

seen to decrease as the rate of wind movement is increased. Now let a line bisecting the right angle at o cut oy at c. The ordinate from o_1t_1 passing through c will give a value of w which may very conveniently be used as the unit. Now since $1^n = 1$, the value of y at this point becomes $y = \frac{t - t_1}{1 + c}$; hence $c = \frac{t - t_1}{y} - 1$. By substituting this value of c into the formula with other experimental values of y and w, the value of n can be obtained readily. A more general

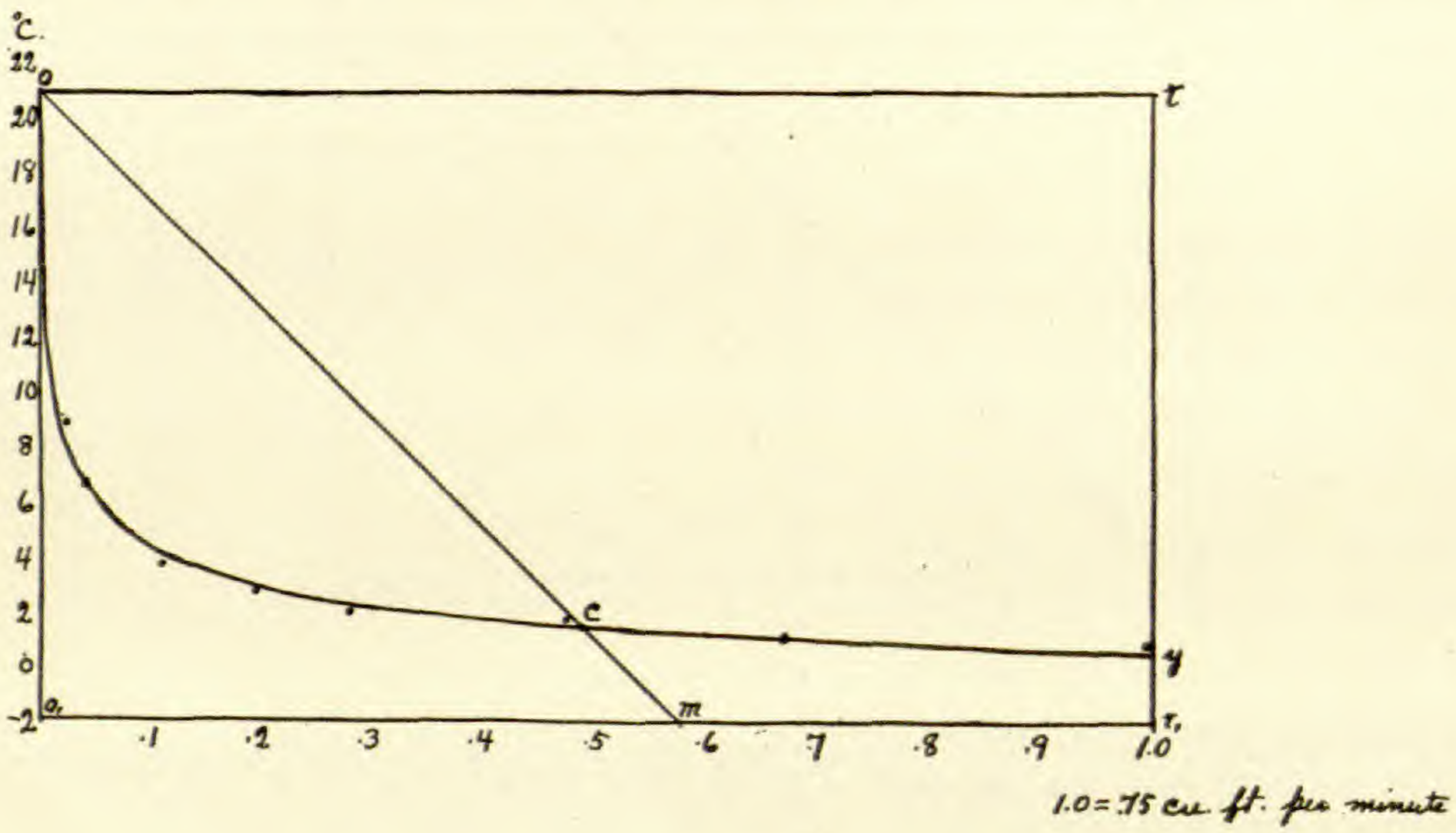

FIG. 3

method of finding the value of n is by the elimination of c, as follows: Going back to the original formula and solving for cw^n, we find that: $cw^n = \frac{t - t_1}{y} - 1 = p$; likewise $cw_1^n = \frac{t - t_1}{y_1} - 1 = p_1$; dividing $\left(\frac{w}{w_1}\right)^n = \frac{p}{p_1}$;

whence $n = \dfrac{\log \frac{p}{p_1}}{\log \frac{w}{w_1}}$.

Knowing the value of n, we can readily find c by the formula $c = \frac{p}{(w)^n}$. The first method for finding the value of c and then of n is quicker and more accurate when as many as four or five experimental points on the curve oy are known, thus enabling one to

locate the point *c* with reasonable accuracy. When only two or three points are known, however, the second method should be used. By the first method, when 0.485 cu. ft. of air per minute was the unit of wind movement, the values of *c* and *n* were found to be 5.706 and 0.5 respectively. With these data the complete theoretical curve *oy* may be calculated and plotted, and the provisional curve used in finding the point *c* corrected and extended.

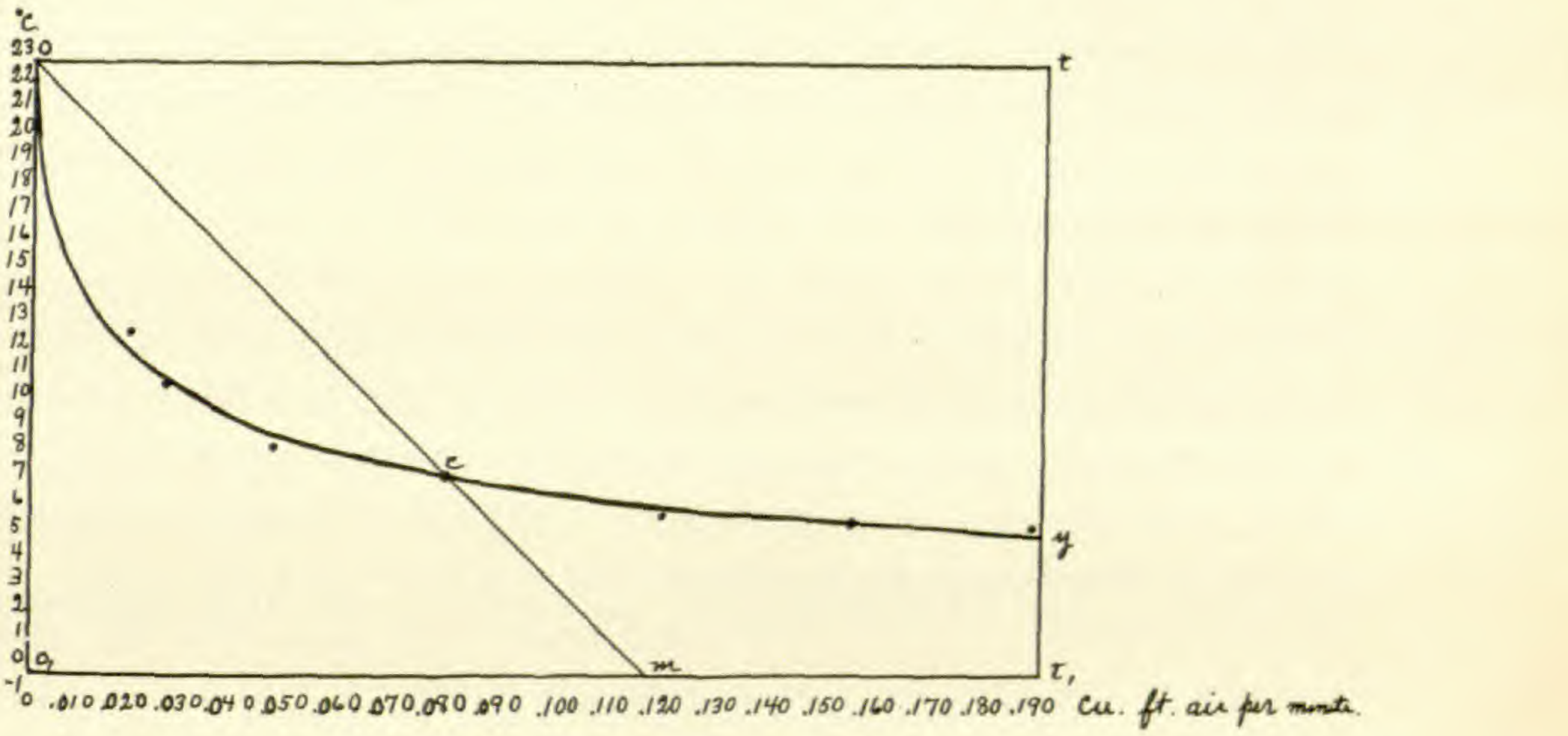

FIG. 4

In fig. 3 the complete curve given is the calculated theoretical curve, and the dots show the close adaptation of the experimental data to it. The agreement appears almost strikingly complete.

Fig. 4 gives an experiment of a similar nature in which $n = 0.58$ and $c = 2$. In this figure the wind movement is drawn on a larger scale than in fig. 3. As will be seen later, the factor *c* includes both the corrections for the area and the nature of the evaporating surface, and to adjust the temperature and wind movement factors to each other. The correspondence of the experimental results with the theoretical curve is again quite close, as is shown by the dots.

It now remains to determine the influence of changes in the area of the evaporating surface, assuming that its character remains constant. Changes in the evaporating area will affect both *c* and *n*. As the area at which *c* is determined rises, the value

of c is diminished; and when the evaporating surface practically fills its environment, c approaches zero. On the other hand, as the area at which c is determined is made smaller, the value of c indefinitely increases.

As the evaporating area increases, the value of n increases, until when the evaporating area practically fills its environment, the value of $n = 1$ (that is, the air passing off will be saturated and the evaporation will be in direct linear proportion to the rate of wind movement). Conversely, when the size of evaporating area is diminished, the value of n decreases, until it reaches the vanishing point, when the evaporating area becomes indefinitely small. When n becomes zero, the value of w^n will equal 1, regardless of the numerical value of w, since any number raised to the zero power equals one. In other words, when the evaporating area is indefinitely small, it cannot measurably increase the humidity of the surrounding air; hence changes in wind movement would have no effect upon the rate of evaporation. Instead of c we may therefore write $\frac{c}{a}$, and for n write $\frac{a}{k+a}$, in which a is any area expressed in terms of the area used when c was determined, and k is a constant. The formula then becomes $y = \frac{t - t_1}{1 + \frac{c(w)^{\frac{a}{k+a}}}{a}}$. Again, if the wind movement used when c and k were determined be made unity, and other wind movements be expressed in terms of it, since 1 raised to any power is 1, the formula for that wind movement becomes $y = \frac{t - t_1}{1 + \frac{c}{a}}$, from which the value of c can readily be calculated from any experimental value of y. This statement of the formula can also be used in experiments in which the wind movement is not varied, and may hence be considered as unity. The details of such an experiment are given in table I, where $c = 0.984$, $w = 1$, $a = 1$, and $a_1 = 1.654$. Since $w = 1$ throughout, the determination of k was unnecessary.

Table II gives another experiment of a similar nature, in which the wind movement was 91.43 liters of air per hour throughout, which

TABLE I

EVAPORATION EXPERIMENT WITH ATMOMETER

Temperature outside	Dewpoint outside	Dewpoint of escaping air	Evaporating surface	Found y	Calculated y	Difference
27.0.....	0	13.8	45.8 sq. cm. = 1	13.8	13.5	−0.3
26.0.....	0	13.2	45.8 sq. cm. = 1	13.2	13.0	−0.2
26.0.....	0	12.8	45.8 sq. cm. = 1	12.8	13.0	+0.2
25.9.....	0	12.8	45.8 sq. cm. = 1	12.8	13.0	+0.2
	Average		.....	13.2	13.1	−0.1
27.0.....	0	16.2	75.47 sq. cm. = 1.645	16.2	16.8	+0.6
26.0.....	0	16.2	75.47 sq. cm. = 1.645	16.2	16.2	0
26.1.....	0	15.8	75.47 sq. cm. = 1.645	15.8	16.2	+0.4
26.1.....	0	16.0	75.47 sq. cm. = 1.645	16.0	16.2	+0.2
	Average		.....	16.1	16.4	+0.3

is here used as unity. The other constants were as follows: $c = 1.257$, $a = 42.04$ sq. cm., here taken as unity, and $a_1 = 75.47$ sq. cm. $= 1.7954$.

There remains to show the details of an experiment in which both the area and wind movement were varied. Here $a = 52.98$

TABLE II

EVAPORATION EXPERIMENT WITH ATMOMETER

Temperature outside	Dewpoint outside	Dewpoint of escaping air	Evaporating surface	Found y	Calculated y	Difference
21.7.....	1.0	10.6	1	9.6	9.2	.....
21.5.....	1.0	10.2	1	9.2	9.1	.....
21.2.....	1.0	10.0	1	9.0	8.9	.....
21.0.....	1.0	10.4	1	9.4	8.9	.....
20.9.....	1.1	10.0	1	8.9	8.8	.....
20.8.....	1.1	10.1	1	9.0	8.7	.....
20.8.....	1.2	10.2	1	9.0	8.7	.....
20.7.....	2.2	11.2	1	9.0	8.2	.....
20.7.....	2.3	10.5	1	8.2	8.1	.....
20.7.....	2.2	9.8	1	7.6	7.6	.....
	Average		.....	8.9	8.6	−0.3
21.7.....	2.2	13.6	1.7954	11.4	11.5	.....
21.6.....	2.3	13.5	1.7954	11.2	11.4	.....
21.4.....	2.3	13.6	1.7954	11.3	11.2	.....
21.0.....	2.2	12.7	1.7954	10.5	11.0	.....
21.0.....	2.2	12.8	1.7954	10.6	11.1	.....
	Average		.....	11.0	11.2	+0.2

sq. cm., taken as the unit of area, $a_1 = 75.47$ sq. cm. $= 1.42$, $k = 3.546$, $c = 1.076$; $w = 91.43$ liters per hour, here used as unity, and $w_1 = 165.62$ liters per hour $= 1.816$.

The calculated and experimental results again agree so closely that the conclusion seems justified that they are within the limits of experimental error.

TABLE III

EVAPORATION EXPERIMENT WITH ATMOMETER

Temperature outside	Dewpoint outside	Dewpoint of escaping air	Evaporating surface	Wind movement	Found y	Calculated y	Difference
25.2.....	2.2	13.0	1	1.816	10.8	10.3	.....
25.1.....	2.2	12.7	1	1.816	10.5	10.3	.....
25.0.....	2.2	12.4	1	1.816	10.2	10.2	.....
24.9.....	2.2	12.3	1	1.816	10.1	10.2	.....
	Average		.....	.....	10.4	10.3	−0.1
25.3.....	1.0	13.0	1	1	12.0	11.7	.....
25.4.....	1.0	12.9	1	1	11.9	11.8	.....
25.4.....	0.9	12.7	1	1	11.8	11.8	.....
25.4.....	1.1	12.6	1	1	11.5	11.7	.....
25.4.....	1.0	12.4	1	1	11.4	11.7	.....
	Average		.....	.....	11.7	11.7	0.0
27.0.....	1.1	15.2	1.42	1	14.1	14.7	.....
26.8.....	1.2	15.2	1.42	1	14.0	14.6	.....
26.6.....	1.2	14.8	1.42	1	13.6	14.5	.....
26.4.....	1.1	16.0	1.42	1	14.9	14.4	.....
26.2.....	1.0	15.2	1.42	1	14.2	14.3	.....
26.1.....	1.0	15.3	1.42	1	14.3	14.3	.....
	Average		.....	.....	14.2	14.4	+0.2
26.0.....	1.2	14.2	1.42	1.816	13.1	13.1	.....
25.8.....	1.3	14.4	1.42	1.816	13.1	12.9	.....
25.4.....	1.4	13.6	1.42	1.816	12.2	12.6	.....
25.3.....	1.4	13.8	1.42	1.816	12.4	12.6	.....
	Average		.....	.....	12.7	12.8	+0.1

An examination in detail of the completed formula will show whether it will continue to appear rational when the several variables concerned are carried to their extreme limits. As t increases, y will increase indefinitely. As t decreases, y will decrease and vanish when t falls to t_1, or to absolute zero. As t_1 increases, y decreases and vanishes when t_1 reaches t. As t_1 decreases, y increases until t_1 reaches absolute zero, at which the size of y will depend on t, a, and w only. As a increases, the value

of the expression $\frac{c(w)^{\frac{a}{k+a}}}{a}$ decreases, until when a is indefinitely large it vanishes; hence the value of y will equal $t-t_1$, that is, the air will be saturated for the temperature t. At the same time the expression $\frac{a}{k+a}$ will approach 1; that is, as the size of the evaporating area increases in relation to its environment, the more dependent is the rate of evaporation upon the wind movement. On the other hand, if a decreases, the value of the expression $\frac{c(w)^{\frac{a}{k+a}}}{a}$ increases and approaches infinity, at which point y would equal zero. At the same time the expression $\frac{a}{k+a}$ will approach zero. In other words, when the evaporating area decreases, the rate of evaporation depends less and less upon the wind movement. As w increases, the value of y will decrease, and vanish when w reaches infinity. As w decreases, y will increase; and when w becomes zero, the value of y will equal $t-t_1$, that is, the air will become saturated for temperature t. It thus appears that the formula remains rational when any of its variables are projected to their extreme limits. Moreover, it appears to correspond, within allowable limits of experimental error, with the results obtained throughout the range of conditions covered by the work of the writer. This range does not include temperatures either above the boiling point or below the freezing point of water. Disturbing factors which might be introduced at those critical stages in the physical condition of water were not investigated, inasmuch as they would practically never be reached in investigations concerned with the transpiration of living plants.

Fig. 5 gives the results of an experiment made upon a potted alfalfa plant. The temperature was raised artificially by means of an electric current passed through a coil of nicrome wire. This was wrapped around the cylinder into which the branches were inserted from below through openings in the stopper. The opening through the stopper was sealed around the stems with low melting point paraffin. The temperature was controlled by regulating the current passing through the resistance wire by means

of a thermostat. Throughout this experiment the air stream was constant at 70.32 liters per hour, and the dewpoint of the exterior air was 4.8° C. The range of temperature was 17.8–36.1°. The range in the value of $t-t_1$ was 18.3°. The lines *ot* and ot_1 give the values of t and t_1 respectively. The line *oy* gives the calculated value of y where z [formula $y=z\ (t-t_1)$] equals 7.45 per cent. The dots show the agreement of the experimental with the calculated value of y.

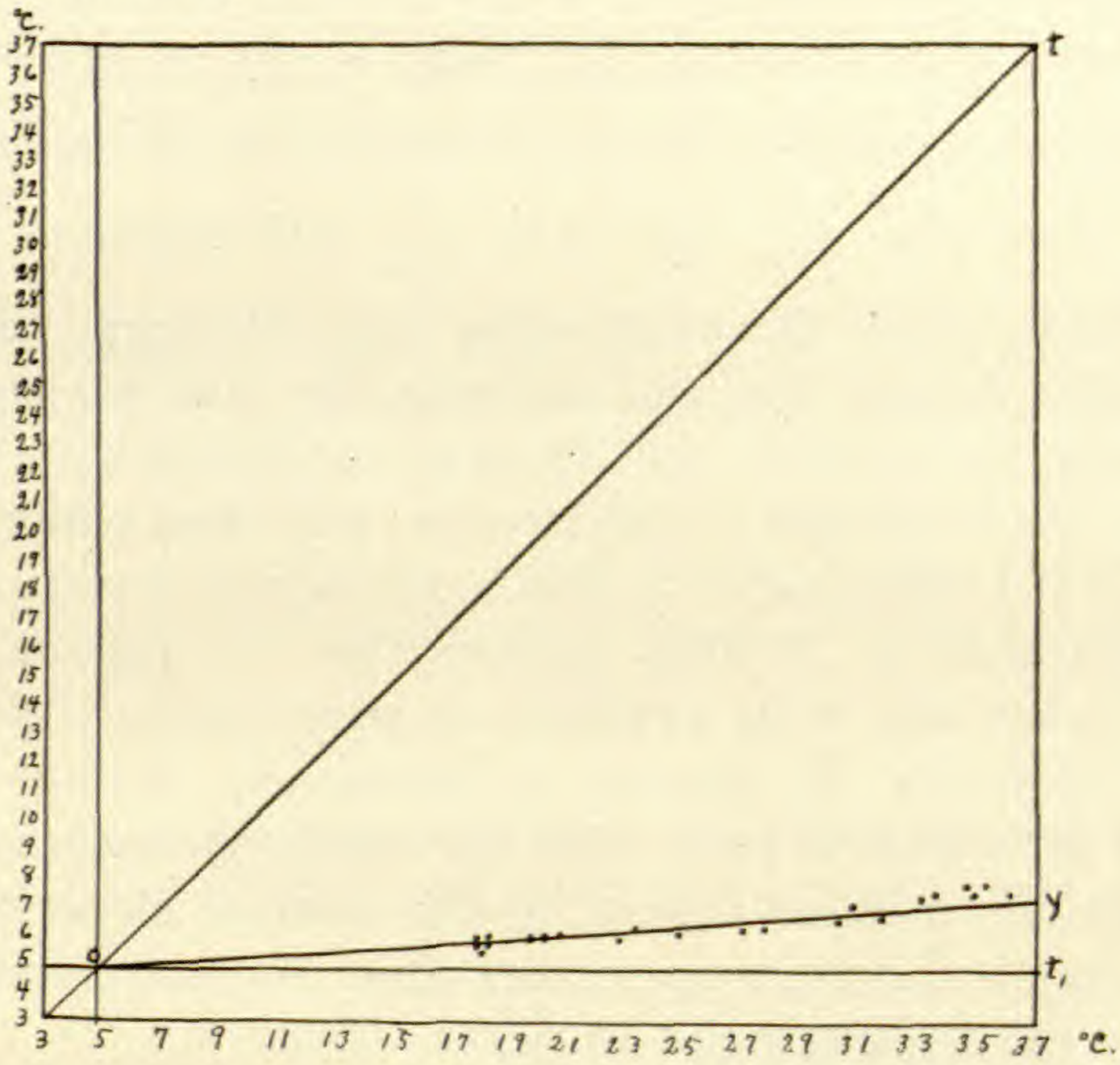

FIG. 5

This figure seems to indicate that under temperature changes of the magnitude here given, the plant acts essentially as a physical evaporating surface. On the other hand, when the relative humidity of the external air is varied, there appears to be a very decided response on the part of the plant, as is shown in the following experiment.

On March 14, 1914, a potted alfalfa plant was taken from the highly humid atmosphere of the greenhouse and brought to the much drier atmosphere of the laboratory, where the light also was not so intense as in the greenhouse. The stems of this plant were sealed into the evaporation cylinder as described (of course

TABLE IV

Time	Temperature		Liters air per hour	Relative humidity		Dewpoint found (x)		Dewpoint calculated inside (y)	Found divided by calculated
	Outside	Inside		Outside	Inside	Outside	Inside		
1:44	18.0	18.3	95	25	79	−3.5	14.4	13.9	1.04
1:47	*a* 18.0	18.1	95	25	77	−3.0	13.8	13.9	0.99
1:56	18.0	17.8	95	25	76	−3.0	13.3	13.3	1.00
2:02	18.0	17.6	95	25	73	−3.0	12.4	13.5	0.92
2:19	*b* 18.1	17.2	95	?	68	?	10.8	.....	.....
2:36	*c* 18.2	16.0	190	?	54	?	6.1	.....	.....
2:53	*d* 18.2	16.6	95	?	66	?	9.9	.....	.....
	2:55 normal air								
3:02	*e* 18.2	16.8	95	24	71	−3.4	11.2	16.1	0.70
3:14	*f* 18.2	16.6	190	24	64	−3.4	9.4	13.5	0.70
	3:16 moist air								
3:36	18.2	18.0	95	79	91	14.3	16.4	17.3	0.95
3:44	*g* 18.2	18.2	95	81	93	14.7	17.0	17.5	0.97
3:54	*h* 18.3	18.2	190	82	94.2	15.1	17.2	17.2	1.00
3:58	18.4	18.2	95	82	96	15.1	17.5	17.3	1.01
4:04	*i* 18.4	18.3	95	82	97	15.1	17.8	17.7	1.01
	4:05 normal air								
4:09	18.4	18.1	95	28	95	−1.3	17.2	14.2	1.21
4:12	18.4	17.9	95	26	91	−2.3	16.3	13.9	1.17
4:16	18.4	17.8	95	26	85	−2.3	15.1	13.8	1.09
4:19	*j* 18.4	17.7	95	26	85	−2.2	15.0	13.7	1.10
4:24	18.5	17.6	95	26	83	−2.7	14.5	13.5	1.07
4:26	18.6	17.5	95	25	83	−2.6	14.4	13.5	1.07
4:29	18.6	17.4	95	25	81	−2.6	13.9	13.4	1.04

without removing them from their own roots growing in the pot), a current of air was drawn over them, and successive readings made, as shown in table IV.

Since the area of leaves was not changed in table IV, the simpler form of the formula $y=\frac{t-t_1}{1+c(w)^n}$ could be used; c was taken as 0.25 and n as 0.93. This high value of n is in keeping with the fact already mentioned, that when the evaporating area is large in comparison with its environment, the value of n will be large. Sixty-two leaves, having an area of approximately 200 sq. cm., were on the stems included within the cylinder. Thus it may be noted that the value of y for the slower wind movement averaged 80 per cent of the value of $t-t_1$. In this experiment both the wind movement and the humidity of the air passed over the plant were varied. The latter was accomplished by (1) using the normal air of the room, (2) first passing the air through tubes of phosphorous pentoxide and then over the plant, and (3) passing over the plant a controlled mixture of saturated and normal air. It is not assumed that the air passed over the phosphorous pentoxide was absolutely dry, but its dewpoint was so low that it could not be detected by the ether-cooled mirror, which could be easily read down to -6 or $-7°$ C.

For the purpose of discussion the experiment may be divided into eight periods, as follows:

(*a*) 1:44–2:07, normal air, slow wind
(*b*) 2:07–2:32, dry air, slow wind
(*c*) 2:32–2:43, dry air, fast wind
(*d*) 2:43–2:53, dry air, slow wind
(*e*) 2:53–2:59, normal air, slow wind
(*f*) 2:59–3:16, normal air, fast wind
(*g*) 3:16–3:45, moist air, slow wind
(*h*) 3:45–3:55, moist air, fast wind
(*i*) 3:55–4:05, moist air, slow wind
(*j*) 4:05–4:29, normal air, slow wind

First compare the column showing the outside temperature with that exhibiting the temperature within the cylinder and measured by a thermometer having its bulb among the leaves. The temperature within the cylinder began 0.3° C. above that

outside, but slowly fell throughout periods *a*, *b*, and *c*, reaching its lowest point in the later period of dry air and high wind movement when it was 2.2° below that of the outside air. When in period *d* slow wind movement was used, the cylinder temperature rose 0.6°. In period *e*, when normal air was used, the cylinder temperature rose 0.2°. In *f*, when fast wind was again used, the temperature dropped 0.2°. When high humidity air was used in periods *g*, *h*, and *i*, and the transpiration was very low, the temperature rose to within 0.1° of that of the external air, but during period *j*, when normal air was used and the evaporation increased, the cylinder temperature steadily fell, until at the end of the experiment it was 1.2° below that of the exterior air. We thus have a definitely measurable cooling effect of evaporating leaves upon the air which passes over them.

In order to study the physiological response of the plant foliage as an evaporating surface, the column giving the dewpoint inside (the dewpoint of the air after it has passed over the plant) may be compared with the next column, which shows the calculated values, considering the leaves as physical evaporating surfaces and using the constants already given. To facilitate this comparison the last column is added to give the ratio of the found to the calculated dewpoint.

It is estimated that the time required to bring the plant from the greenhouse and instal it in the transpiration apparatus and obtain the first reading was about half an hour. Suppose the evaporating condition at this time to be 104 (see last column of table). By the end of period *a*, with the plant exposed to the drier laboratory air (23 minutes), this had dropped to 92. During periods *b*, *c*, and *d* (46 minutes) the plant was exposed to air which had passed over phosphorous pentoxide. Although the comparison of the calculated with the found results cannot be made here, it may be observed that the dewpoint during period *d* was lower than during period *b*, which indicates a still further restriction of evaporation. When the plant was put back on normal air in period *e*, it was found that this restricting influence had cut down the found dewpoint to 70 per cent of the calculated value, and that this was maintained throughout the 23 minutes of periods *e*

and *f*. When, however, a stream of very moist air (relative humidity 79–82 per cent) was used, the plant again responded, and within 20 minutes (reading at 3:36 P.M.) had reached an evaporation rate of 95 per cent of the calculated value. This increase continued until at 4:04 (28 minutes later) it was 101. When the shift was again made to normal air (at 4:05) there was exhibited at 4:09 (4 minutes later) a special emphasis of this high transpiring condition of the plant, since it gave a reading of 121 per cent of the calculated value. The effect of the drier normal air soon became apparent in the steady fall of this ratio, which at 4:29, or 24 minutes after removal of the moist air, had reached a value of 104, which was identical with its value at the beginning of the experiment, when it had likewise been removed from the saturated air of the greenhouse some 25 or 30 minutes previously.

The response of the plant to the humidity of the air is strongly indicated here. This response is rapid but not immediate. In looking over a number of experiments similar to the one described, it appears that when the humidity is suddenly changed but remains constant thereafter, complete adjustment is usually attained in 30–35 minutes.

It may be recalled that in the experiment involving changes in temperature only (fig. 5), the plant leaf acted approximately as a physical evaporating surface. This was true in spite of the fact that the change in the temperature of the air passing over the plant also involved a considerable change in its relative humidity. Its actual dewpoint, that is, the water contained per liter, remained constant. At the higher temperatures the actual dewpoint of the air after passing over the plant was always higher than at the lower temperatures; that is, the leaves were evaporating more into an air of higher dewpoint than at the lower temperatures. This fact approximately offsets the effect of the greater water demand made upon the plant by the greater transpiration at the higher temperature, and gives a result which is very close to the behavior of a physical surface. When, however, the water content, that is, the dewpoint of the incoming air, is increased (other conditions remaining the same), the evaporation, or the water demand upon the plant, is strongly reduced.

We may infer from this that the guard cells of the stomata become more turgid, making these openings larger, and thus increase the evaporation coefficient of the surfaces.

The use of this formula in interpreting the results of comparative tests of the transpiration of different varieties or species of plants may be illustrated by two examples. The first may be taken from seven experiments made upon a peach tree (*Prunus persica*) and a large greasewood shrub (*Covillea mexicana*), which were growing in close proximity upon well cultivated and watered soil on the grounds of the University of Arizona. The data of this experiment are given in table V, which includes the temperature within the cylinder, the dewpoint of the external air, the dewpoint of the air coming out of the cylinders, the relative humidity of the same, and the number of square centimeters of leaves used. There is then placed in the last column a calculated "standard dewpoint," which is found by means of the formula, and is the dewpoint of the outgoing air which would have been given by a physical surface of the same evaporating potentiality as the given leaves (at the time when the experiment was made), but having an area of 100 sq. cm., a cylinder (air) temperature of 30° C., and an external dewpoint of 10° C. The wind movement in all cases was the same, being approximately 85 liters per hour. It will be noted in column *e* that the dewpoint of the escaping air from the greasewood cylinder was higher three times and lower four times than the dewpoint of the air escaping from the peach cylinder. When, however, each is calculated to the standard dewpoint, that of the air from the greasewood cylinder was always higher. It is surprising to find that the leaves of the greasewood (a strictly xerophytic plant) show such a uniformly higher transpiration rate than those of the peach (a deciduous mesophyte). It is interesting to note, therefore, that, whereas 100 sq. cm. of the greasewood leaves weighed 3.93 gm., a similar surface of peach leaves weighed only 1.92 gm. When the calculations were based upon equal weights of herbage, the peach showed an equally uniform greater transpiration per 100 gm. green weight foliage. The greasewood experimented upon was growing in the orchard where it received an abundance of irrigating water. Its leaves

TABLE V

Date (*a*)	Plant (*b*)	Temperature in cylinder (*c*)	Dewpoint of external air (*d*)	Dewpoint of escaping air (*e*)	Relative humidity of escaping air (percentage) (*f*)	No. of sq. cm. of leaves used (*g*)	Standard dewpoint (*h*)	Excess of greasewood
May 21...	Greasewood.....	31	−4.3	21.9+	60	149.7	23.2	+4.3
	Peach.....	30	−4.3	18.5	53	249.4	18.9	
May 22...	Greasewood.....	33	+0.9	24.2+	61	122.2	23.7	+1.3
	Peach.....	29	+0.9	22.7	70	211.5	22.4	
May 22...	Greasewood.....	35	−2.7	23.5−	54	100.3	23.9	+1.9
	Peach.....	33	−2.7	24.3	62	206.5	22.0	
May 23...	Greasewood.....	34	−1.7	29.8−	81	178.8	26.1	+0.1
	Peach.....	34	−1.7	30.1	82	204.8	26.0	
July 1.....	Greasewood.....	38	−6.7	28.4+	60	106.5	25.5	+6.2
	Peach.....	38	−6.7	19.4	36	160.9	19.3	
July 5.....	Greasewood.....	39	+6.1	26.7−	52	94.8	22.8	+5.7
	Peach.....	42	+6.1	28.2	50	293.1	17.1	
July 12....	Greasewood.....	36	+9.5	26.9−	58	64.9	24.9	+4.6
	Peach.....	37	+9.5	27.0	59	116.1	20.3	

were very thick, green, and luxuriant, being markedly different from those growing on the dry mesa a few hundred yards distant. A single experiment comparing the orchard greasewood with that of the mesa gave a standard dewpoint 2° higher for the former. This indicates that its leaves had undergone a modification which favored a greater transpiration rate.

As an example of the use of this method in the study of the comparative water loss from different varieties of a single species, a set of eight experiments extending over a period of about two weeks may be given. These were upon two pure lines of alfalfa nos. 91 and 17. The results are collected in table VI, which has the same arrangment of material as table V. The dewpoint of the escaping air was greater for the cylinder covering branches of race no. 91 three times and less four times, but when calculated to the same standard dewpoint as used in table V, it was greater from race no. 91 for six out of the seven experiments made. These results, which would appear hopelessly confusing at first, when calculated to the standard dewpoint, seem to indicate with a fair degree of definiteness that the leaves of race no. 91 offered less hindrance to evaporation than did those of race no. 17. Here again it was the thicker leaves which gave the greater transpiration rate, since the average weight per 100 sq. cm. of race no. 91 was 2.57 gm., whereas that of race no. 17 was only 2.29 gm.

In comparing tables V and VI it will be noted that the differences in the standard dewpoints of the same species or variety for different days was often as great as or greater than the differences between the species or varieties being compared on the same day. In spite of this, however, the differences between the species or varieties were so constant that one cannot but believe they were real and significant. The differences in the standard dewpoints for different days must be sought therefore in some common factor which has influenced both types alike.

Such a factor, as shown in table IV, is the dewpoint of the external air. In examining tables V and VI it will be noted that whereas high standard dewpoints are perhaps more frequently than otherwise accompanied by high external dewpoints, this is

TABLE VI

COMPARISON OF TRANSPIRATION OF TWO PURE RACES OF ALFALFA

Date	Race no.	Temperature in cylinder	Dewpoint of external air	Dewpoint of escaping air	Relative humidity of escaping air	No. of sq. cm. of leaves	Standard dewpoint	Excess of race no. 91
May 12...	91.....	31	−0.2	19.4+	52	91.7	23.0	+1.6
	17.....	31	−0.2	16.2	43	82.8	21.4	
May 13...	91.....	27	0.0	17.2+	57	97.2	22.9	+2.1
	17.....	28	0.0	15.3	48	101.9	20.8	
May 14...	91.....	22	+3.0	15.7−	69	77.7	24.4	+0.5
	17.....	23	+3.0	15.9	66	79.6	23.9	
May 14...	91.....	25	−1.1	16.9−	66	79.4	24.5	−0.7
	17.....	24	−1.1	17.4	64	87.9	25.2	
May 15...	91.....	26	−1.0	17.4+	58	71.4	25.0	+0.2
	17.....	22	−1.0	16.0	70	99.5	24.8	
May 19...	91.....	28	−1.3	19.6−	61	83.3	25.0	+0.6
	17.....	26	−1.3	20.6	71	156.3	24.4	
May 20...	91.....	30	+2.4	23.9−	72	75.5	26.5	+0.5
	17.....	30	+2.4	24.6	75	103.4	26.0	

by no means always the case. There are other factors, for instance, such as the amount of light reaching the plant on more or less cloudy days, the age of the plants, and moisture content of the soil, all of which certainly affect the condition of the stomata and hence the evaporating efficiency of the leaves. These would, moreover, affect both types approximately alike.

Another disturbing factor, which would not equally affect the plants, is the area of foliage of each inclosed in the evaporating chamber. If such an amount of foliage is inclosed in one cylinder as to give its outgoing air a markedly higher dewpoint than that of the other cylinder, the evaporating rate per unit surface will be cut down by the greater relative humidity in the cylinder. The water demand upon a given area of the leaves will be reduced, and they will respond by opening wider their stomata. This will give the leaves of this cylinder a greater standard dewpoint than will be found for the leaves in the cylinder having the less surface exposed. In practical work, therefore, it is necessary either to inclose in each cylinder an approximately equal area of leaves (which is extremely difficult), or to so vary the wind movement as to have the dewpoint of the air, coming off each cylinder, approximately equal. This can be easily accomplished in the apparatus used by the writer, by pinching down the air supply tube of one or the other cylinders with a screw clamp.

Summary

1. As a result of evaporation experiments carried out by means of a porous cup atmometer inclosed in a glass cylinder of one liter capacity, through which an air current is passed, an evaporation formula is offered which may take any of the following forms:

$$\text{(a) } y=z(t-t_1);\quad \text{(b) } y=\frac{t-t_1}{1+c(w)^n};\quad \text{(c) } y=\frac{t-t_1}{1+\frac{c(w)^{\frac{a}{k+a}}}{a}}.$$

In these formulae y = rise in the dewpoint of the air caused by the loss of water to it of a given evaporating surface; t = temperature of the air; t_1 = dewpoint of the outside air; z = constant used when the area and wind movement remain constant; n = exponent of w, used when the area remains constant; c = constant coefficient

of w, used either when the area is constant (when a does not appear in the formula) or when a varies and hence appears in the formula; a = area of the evaporating surface which is always expressed as the ratio of the surface exposed to that exposed when c and k were determined; when c and k are determined, the area then used is taken as unity and all other areas expressed in terms of it; k = constant used in the exponent of w to adjust the area unit to the wind movement unit.

2. These formulae appear to be general in type and capable of use in any situation where y is measurable. It is possible that with some modification these formulae may also be of use when y cannot be measured, but where it is possible to measure directly the actual evaporation per unit area.

3. Under temperature changes only, alfalfa leaves appear to act as physical evaporating surfaces.

4. Changes in the dewpoint of the air result in profound changes in evaporating efficiency of leaf surfaces. This is probably a result of the opening and closing of the stomata.

5. It is possible to make use of these formulae in comparing the evaporating efficiency of different species of plants and interpreting results which would otherwise appear hopelessly confusing.

6. Distinct pure races of alfalfa exhibit measurable differences in the rate of evaporation per unit area of their leaves. Such differences may be of economic value in semi-arid or irrigated regions where production depends principally upon the efficiency of the use of the available water supply.

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EARLY DEVELOPMENT OF INOCYBE

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(WITH PLATES XVIII–XXII)

During a collecting trip with Professor ATKINSON at Coy Glen, near Ithaca, New York, on August 25, 1914, we came upon a large quantity of *Inocybe infelix* in all stages of development. At that time Professor ATKINSON was particularly interested in obtaining *Inocybe* material, as he had in mind the publication of a monograph of the genus. He felt that, before the systematic relationship could be determined with absolute certainty, there was need for more morphological work on the developmental stages of these plants. Accordingly he turned over this material to me as a nucleus of a paper on the development of *Inocybe*. Two years later, in July, while collecting fungi in the Adirondacks near Seventh Lake, material of *I. eutheloides* and *I. geophylla* was added, the latter collected by Miss LENA B. WALKER. In the following summer the same locality furnished *I. hystrix*, collected by Professor ATKINSON. The *I. obscura* material came from a hard gravelly path in Coy Glen on September 24, 1916.

All the *Inocybe* forms studied here are ground-inhabiting, and were collected from places where the soil was compact and comparatively poor in humus. The determinations were made by Professor ATKINSON from mature plants springing up from the same mycelium as the young buttons taken for study.[1] It is certainly to be regretted that this paper was not put forward at that time, before the untimely death of Professor ATKINSON, who felt so keenly the need of a closer cooperation of the systematist with the morphologist in working out plant relationships. The

[1] Plants were fixed in the field in medium chromo-acetic acid, care being taken to remove as much of the soil as possible before dropping them into the fluid. The washing afterward removed most of the gritty particles. After dehydration they were cleared in cedar oil and imbedded in 52° paraffine. Sections were cut 5–7 μ in thickness and stained with fuchsin, after having been previously treated with tannic acid. This method had been found to be most satisfactory for photographing other forms of agarics (9), and proved equally efficient in this case.

development proved to be so similar in all the forms studied that it will be unnecessary to describe it separately for each one. Accordingly, a general account of the method of growth will be given and any minor specific variations noted from time to time.

PRIMORDIUM OF BASIDIOCARP.—Very young stages in the development of the fruit body were found in only two of the five species, in *I. hystrix* (fig. 53) and *I. geophylla* (fig. 70). The button of *I. hystrix* measures 0.6×0.35 mm., and is composed of loosely interwoven hyphae ranging from 2 to 3 μ in diameter. In the basal end there is a more deeply staining region, conical in shape, where the elements are considerably thicker. This represents the primordium of the stem whose hyphae, multiplying very rapidly, are advancing into the fundamental tissue above. The young button at the right in fig. 70 of *I. geophylla* is composed of interlaced fibers 1–2 μ thick in the denser stem primordial region inside and 2–4 μ on the outside, where apparently they are disintegrating and wearing off through the contact with the soil.

DIFFERENTIATION OF PILEUS AND STEM PRIMORDIA.—The dome-shaped stem primordium at the base of the fruit body continues to advance upward within the fundamental tissue. After a time the hyphae in the apical region, which have been branching profusely and twining in and out through the interstices of the young button, change their general upward direction and radiate outward on all sides (fig. 55). Thus the pileus fundament appears in the form of a more or less globular cap, separated by an annular furrow from the primordium of the stem below (figs. 1, 2, 21, 39, 54, 55, 71). This method of progressive differentiation of first the stem and then the pileus from the apex of the former has previously been described by MÖLLER (10) for *Rozites gongylophara*, by ATKINSON (8) for *Lepiota cristata* and *L. seminuda*, by DOUGLAS (9) and SAWYER (12) for species of *Cortinarius*, by SAWYER (11) for *Pholiota squarrosa*, *P. flammans*, and *P. adiposa*, and by WALKER (13) for *Tubaria furfuracea* among the endogenous forms.

BLEMATOGEN.—The remnant of the original fundamental tissue, still enveloping the fundaments of pileus and stem, becomes the true "universal veil," or, as we shall call it, the blematogen. This name was proposed by ATKINSON (2, 3) because of the indefi-

niteness in the application of the former term by the earlier writers. Although there are minor differences in its character and considerable variation in its final disposition, it has been found to be a homologous structure in all endogenous forms. In the *Amanita* type, as illustrated by *Amanitopsis vaginata* (4), the blematogen is separated from the pileus by a clean-cut cleavage layer and forms the volva. In all the other forms it becomes more or less "concrete" with the pileus, so that in the majority of cases it is impossible to distinguish where the pileus leaves off and the blematogen begins. In *Agaricus* forms (1, 2, 6) considerable blematogen enters into the formation of the cortex of the pileus. In others it becomes the cuticle (*Cortinarius anfractus* and *C. armillatus*, 9). Often it is shed in the form of scales (*Cortinarius distans*, 9) which may be mealy or powdery (*Lepiota cristata*, 8, *Coprinus micaceus*, 7). In *Pholiota* (11) scales are formed from the blematogen which partly gelatinize. Frequently fibrillose scales of blematogen persist in the mature plants, as in *Lepiota clepeolaria* (5) and *Coprinus comatus* (7).

In four of these species of *Inocybe* the blematogen is a very delicate structure, which very soon disappears from the surface of the pileus. In *I. obscura* (pl. XVIII), *I. infelix* (pl. XIX), *I. entheloides* (pl. XX), and *I. hystrix* (pl. XXI) the pileus seems to expand through the blematogen to the surface, where it gives off the characteristic silky *Inocybe* fibrils. There is a gradual transition in the size of the hyphae from the inner ones, about 2 μ in diameter in the denser region, to the loose radiating ones on the outside which measure 4–6 μ in thickness. These on the outside take the stain more deeply than those within, and behave generally as disintegrating hyphae.

The condition is slightly different in *I. geophylla*, which belongs to a section of the genus characterized by a pileus which does not crack and which is covered with interwoven fibrils. That these interwoven fibrils are of blematogenous origin seems clear from the fact that they appear in the early stages before the pileus primordium has advanced to the surface of the fruit body (fig. 71). They form a deeply staining thin envelope over the whole of the developing basidiocarp. This envelope might possibly be

considered as a "primary universal veil" or protoblem,[2] such as occurs in addition to the blematogen over the surface of young buttons of *Agaricus campestris*. It does not, however, show as a definite layer in buttons younger than the one shown in fig. 71, although there are hyphae more deeply stained and with larger diameters at the surface in some (fig. 70). The irregularity of the latter would seem to indicate that they are the ends of hyphae undergoing disintegration on account of their contact with the soil. No trace of a protoblem was found in the four other species. This of course may be due to the fact that it was washed off during the rather vigorous treatment necessary to remove the grit particles, but it hardly seems possible that no trace would be left had it existed. The envelope of interwoven fibrils persists in *I. geophylla* (pl. XXII) in the oldest stages collected, and forms here the outer portion of a duplex universal veil such as was described in *Cortinarius anfractus* and *C. armillatus* (**9**). From this loose fibrils arise (fig. 79). Within is a looser, much more delicate tissue with large air spaces. The latter becomes more or less concrete with the pileus at the apex of the fruit body, and shows most clearly between the pileus margin and the stem below the gill cavity.

PRIMORDIUM OF HYMENOPHORE.—Following the differentiation of the stem and pileus primordia, or possibly proceeding at the same time in *I. infelix* (fig. 21), the fundament of the hymenophore makes its appearance (figs. 3, 4, 16, 19, 22, 23, 35, 36, 40, 41, 47, 48, 72, 73). The hyphae in the annular furrow left between the pileus and stem multiply very profusely and turn perpendicularly downward from the more laterally directed fibers of the pileus trama. They become very rich in protoplasm, as shown by their propensity for stain. They are very slender, with more or less pointed ends, so that they are able to push their way easily through the ground tissue. Such a rapid increase in growth here, while practically none is taking place in the blematogen, brings about a stretching and finally a rupturing of the latter tissue, bringing about the formation of the annular cavity (figs. 3, 4). Usually a well defined annular cavity does not make its appearance until the fruit body is slightly older and the palisade layer is organized

[2] See ATKINSON (**2, 3**) for a definition of this term.

(figs. 24, 25, 37, 42, 56, 74). At first the extent of growth of these fibers is very unequal, and in consequence the primordial surface is very uneven. Gradually, however, the ends of the hyphae become more blunt and even, and beginning at the stem a palisade layer commences to extend outward toward the margin of the pileus. The newest elements are always the smallest. These occur nearest the stem where they are also the most crowded. As the palisade layer passes outward into the primordium the elements appear to be less crowded, more irregular, and larger in diameter. The primordium of the hymenophore gradually passes into pileus margin, where the hyphae are strongly hyponastic and grow irregularly into the blematogen over the stem (fig. 67).

ABSENCE OF MARGINAL VEIL.—As the interstitial growth of the hyphae pushes the margin of the pileus outward, the gill cavity becomes larger and larger, and in consequence the blematogen, which lies between the pileus and stem, is constantly reduced. Whatever fibrils or scales are left on the margin of the pileus are remnants of this. No instance could be found which showed clearly any additional growth of fibers from the margin of the pileus or from the stem into the fundamental tissue which lies between these two regions. The pileus margin is strongly hyponastic and its hyphae, curving downward and inward, grow into the blematogen, but do not reach completely across to the stem (figs. 11, 31, 56, 61, 67, 74, 77, 79). The delicate cobwebby fibrils, which cover the gill cavity in young buttons and usually rupture very early (fig. 65), are evidently of blematogenous origin. In the forms studied here there is no marginal veil in the sense of ATKINSON, who considered it a structure at least in part "sui generis" (**2, 3**). This condition is what we should naturally expect, as one of the characteristics of the genus *Inocybe* in the systematic works is the absence of a marginal veil. By the time the period of vigorous lateral expansion of the pileus is reached, scarcely a trace of the blematogen remains (fig. 65).

DEVELOPMENT OF GILLS.—The gills develop in exactly the same manner as has been described previously for all endogenous forms (excepting those of the *Amanita* type), very careful and detailed accounts of which may be found in ATKINSON's later papers (**6, 7**).

As has been shown, the pileus begins its development at the stem and gradually extends its organization into the hymenophore primordium at the margin of the pileus (fig. 67). Growth continues to be centrifugal. The gill ridges start to develop at the stem in regular spaced areas and radiate outward toward the margin. On account of the very great density of the palisade elements and the continuous addition of new fibers by branching of the palisade elements, the surface of the latter is soon thrown out into folds (figs. 18, 38, 66). Multiplication of hyphae nevertheless continues to go on, and with it certain elements in the layer subjacent now begin to elongate rapidly and to carry downward the much crowded palisade layer. Since the growth of these young salients begins at the stem, the gills elongate first in this region. Passing outward the ridges become less developed until they disappear in the even palisade surface (figs. 7–10, 26–30, 42–46, 56–60, 67). Elongation of the elements of the trama continues while the ridges are progressing outward until mature gills, extending from the stem to the pileus margin, are fully developed. As these radiating rows get farther and farther apart, secondary gills form in the same manner between them. Before the basidia mature, certain elements of the palisade layer push out very rapidly and form cystidia (figs. 49, 68, 69), the nucleus of one of which shows plainly in fig. 49. Successive stages in the development of young gill salients are shown in the high-power photographs of *I. entheloides* (figs. 50–52). Occasionally the gill cavity is strongly arched,[3] and the development of the ridges appears to begin on the stem, a circumstance which brings about appearances such as are shown in figs. 43 and 44 in sections cut parallel to the medium axis. In consequence the salients here are cut nearly perpendicular to their direction of growth, and the gill cavity which extends in between them appears as a series of little pockets. A similar appearance at the margin is often caused by cutting perpendicular to the salients on the enrolled edge of the pileus (figs. 14, 15, 34, 64). The development of these plants was not carried beyond the stages represented in figs. 49, 68, and 79, in which well developed cystidia,

[3] See ATKINSON (**6, 7**) for a complete explanation of these appearances.

but as yet only primordia of basidia and paraphyses, had formed on the half mature gill salients.

Summary

1. Basidiocarps are developed from young buttons of fundamental tissue, within which is organized a growing region of very densely interwoven hyphae of small diameters. By progressive growth the stem is first formed, and later, at the stem apex, the pileus fundament, while the ground tissue on the outside becomes the blematogen or universal veil. No marginal veil is formed. The blematogen is finally completely lost or becomes concrete with the pileus.

2. The gills develop as previously described for forms of the Agaricaceae (not of the *Amanita* type). In the furrow between the pileus and stem fundaments a dense irregular hymenophore primordium is formed, which is soon replaced by an even palisade layer. Crowding of the elements in the palisade layer, due to excessive branching, causes the surface to be thrown outward into folds, which are then carried downward by the elongation of hyphae of the layer subjacent to them. The ridges develop from the stem centrifugally in regular radial rows. Later secondary gills form in a similar manner between them.

In conclusion I wish to express my appreciation to Professor W. W. ROWLEE for his kindness in allowing me the use of the photographic apparatus in the College of Arts and Sciences.

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ITHACA, N.Y.

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EXPLANATION OF PLATES XVIII–XXII

Figs. 1–15, 21–34, 39–46, 53–65, 68, 70–81 were taken with a Zeiss horizontal camera and a Spencer Lens Co. 16 mm. objective; the others were photographed by means of a Bausch & Lomb vertical camera and Zeiss lenses.

PLATE XVIII

Inocybe obscura

Figs. 1, 2.—Median sections of two young fruit bodies, showing differentiation within blematogen of stipe and pileus fundaments; ×34.

Figs. 3, 4.—Median and tangential sections of older basidiocarp in which hymenophore primordium and gill cavity have appeared; ×34.

Figs. 5, 6.—Median and tangential sections of fruit body, showing palisade layer; ×34.

Figs. 7–10.—Series of median and tangential sections of a fruit body in which young gill salients have appeared; ×34.

Figs. 11–15.—Median and tangential sections of older fruit body, showing gills more strongly developed; fibrillose hyphae from pileus have replaced blematogen; ×34.

Figs. 16, 19.—Details from figs. 4 and 3 respectively, showing hymenophore primordium; ×158.

Figs. 17, 20.—Details from figs. 6 and 5 respectively, showing crowded palisade layer previous to formation of gills; ×158.

Fig. 18.—Young gill salients; detail from fig. 9; ×158.

PLATE XIX

Inocybe infelix

FIG. 21.—Median section of young fruit body, showing organization of stem, pileus, and hymenophore fundaments within blematogen; ×34.

FIGS. 22, 23.—Median and tangential sections of slightly older basidiocarp in which gill cavity has appeared; ×34.

FIGS. 24, 25.—Median and tangential sections of fruit body in which palisade layer is developing; ×34.

FIGS. 26–30.—Median and tangential sections of fruit body in which gills are beginning to form; ×34.

FIGS. 31–34.—Series of sections from older fruit body with developing gills; ×34.

FIGS. 35, 36.—Details from figs. 22 and 23 respectively, showing hymenophore primordium; ×240.

FIG. 37.—Detail from fig. 25, showing palisade layer; ×240.

FIG. 38.—Detail from fig. 28, showing young gill salients; ×240.

PLATE XX

Inocybe eutheloides

FIG. 39.—Young button in which pileus and stem primordia are outlined within loose blematogen; ×34.

FIGS. 40, 41.—Median and tangential sections of older fruit body, showing growth of pileus into blematogen layer and hymenophore primordium; ×34.

FIGS. 42–46.—Median and tangential sections of fruit body in which gills are forming; ×34.

FIGS. 47, 48.—Details from figs. 40 and 41 respectively, showing hymenophore primordium; ×158.

FIG. 49.—Section of gills of more mature fruit body, showing developing cystidia; ×158.

FIGS. 50–52.—Series of sections from fruit body of figs. 42–46, showing successive stages in formation of gill ridges; ×158.

PLATE XXI

Inocybe hystrix

FIG. 53.—Young button, showing fundamental tissue into which stem primordium is advancing; ×34.

FIGS. 54, 55.—Median sections of two fruit bodies in which pileus fundament is advancing into blematogen; ×34.

FIGS. 56–60.—Series of longitudinal sections, showing development of gill salients; ×34.

FIGS. 61–64.—Series of sections from slightly older fruit body; ×34.

FIG. 65.—Median section from nearly mature fruit body in which pileus has begun to expand; ×34.

FIG. 66.—High power photograph from fruit body of figs. 56–60; ×158.

FIG. 67.—Detail from fig. 61, showing progressive differentiation of hyphae from primordial hymenophore into pileus margin; ×158.

FIG. 68.—Tangential section of fruit body of fig. 65, showing developing cystidia; ×34.

FIG. 69.—Detail from fig. 68; ×158.

PLATE XXII

Inocybe geophylla

FIG. 70.—Longitudinal sections of young buttons; ×34.

FIG. 71.—Median section in which pileus and stem fundaments are outlined within a 2-layered blematogen; ×34.

FIGS. 72, 73.—Median and tangential sections of fruit body in which primordium of hymenophore is appearing; ×34.

FIGS. 74–76.—Median and tangential sections of older fruit body in which palisade layer has become organized and first gill salients are appearing; ×34.

FIGS. 77, 78.—Median and tangential sections of older fruit body; ×34.

FIGS. 79–81.—Older fruit body in which gills are nearly mature and on which cystidia have developed; duplex universal veil still present over whole fruit body; ×34.

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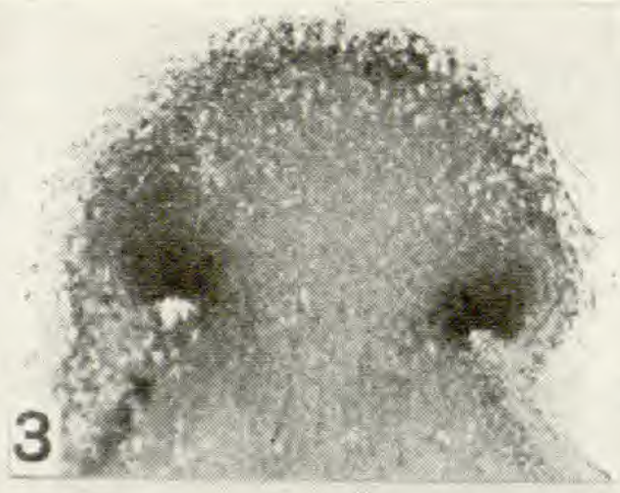
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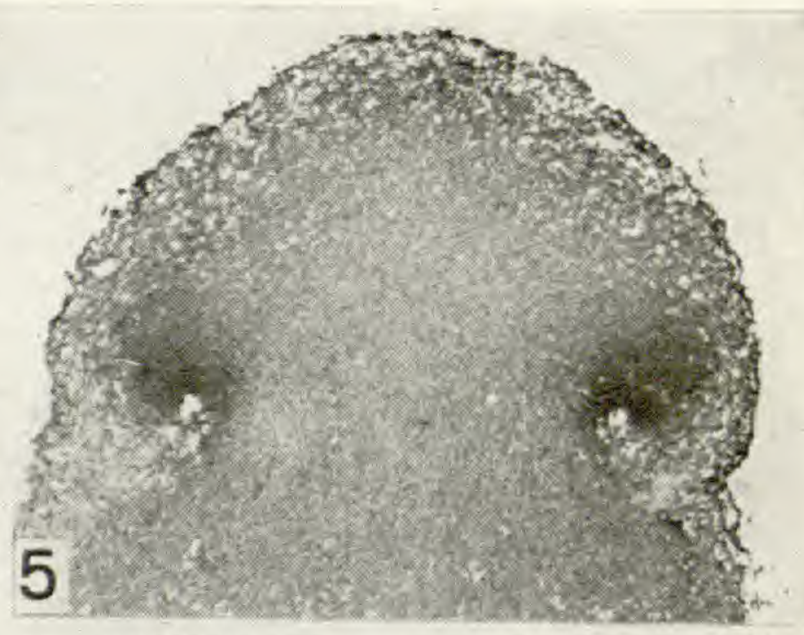
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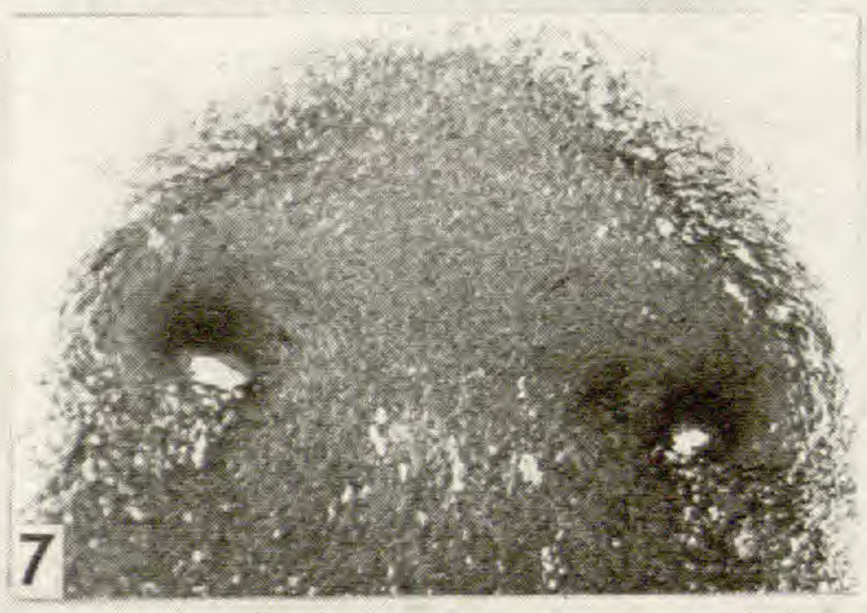
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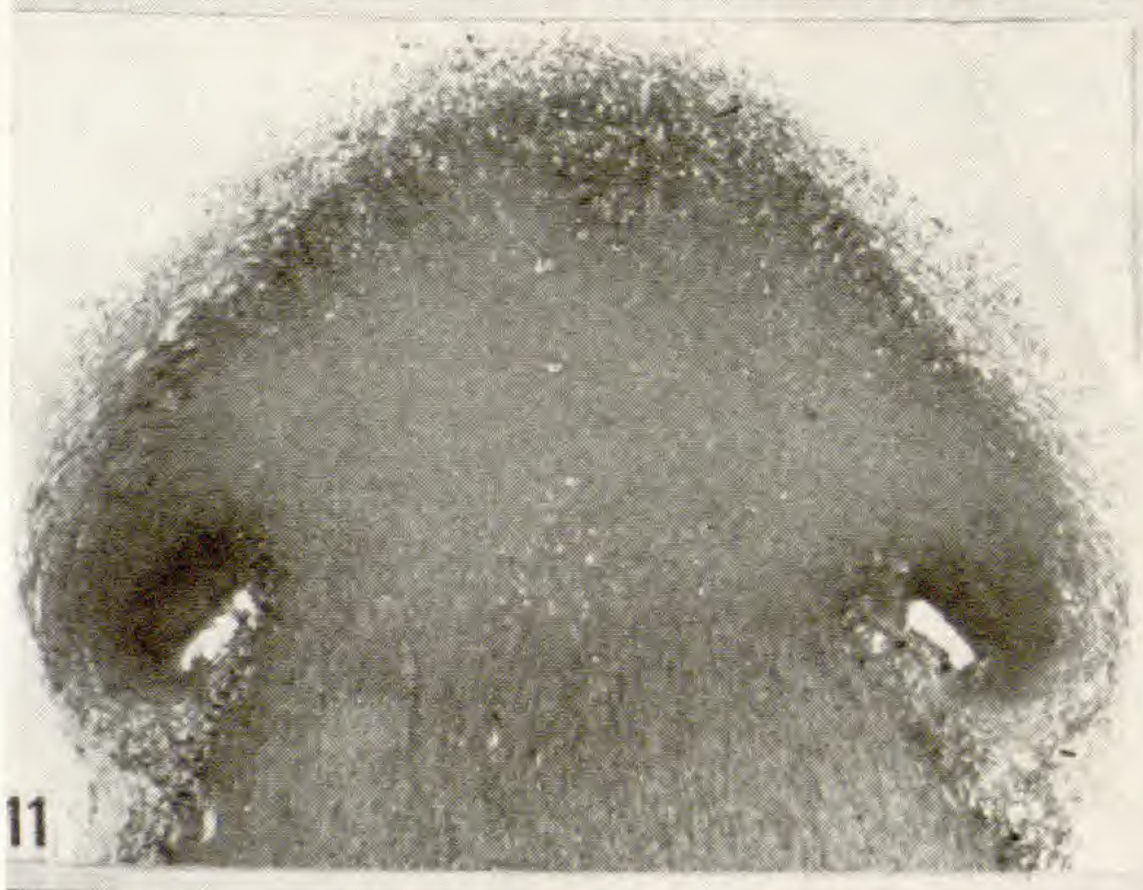
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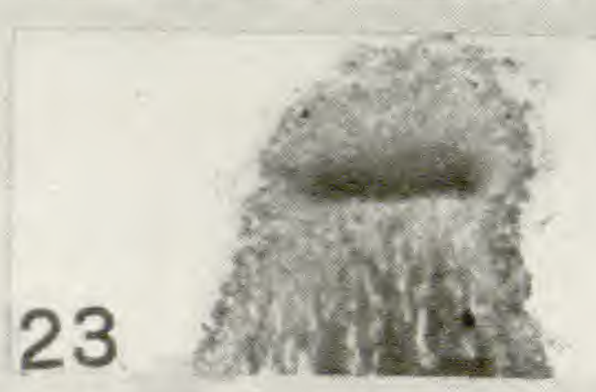
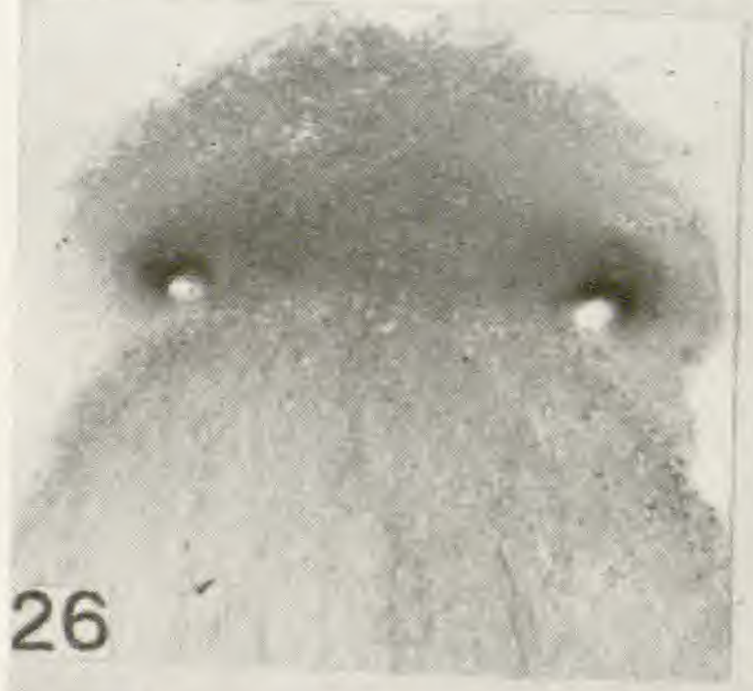
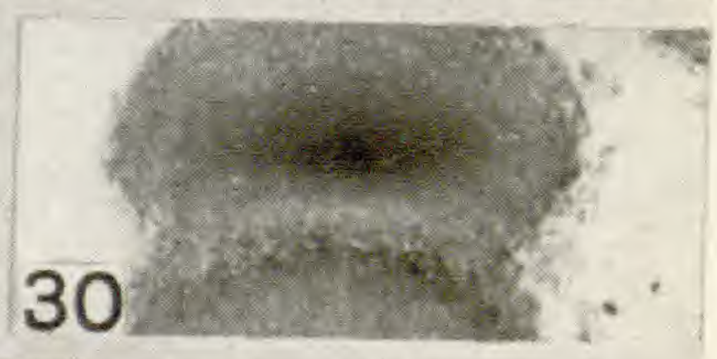
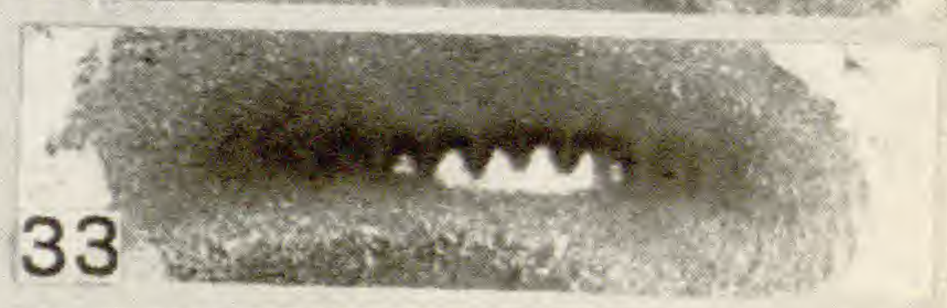
DOUGLAS on INOCYBE

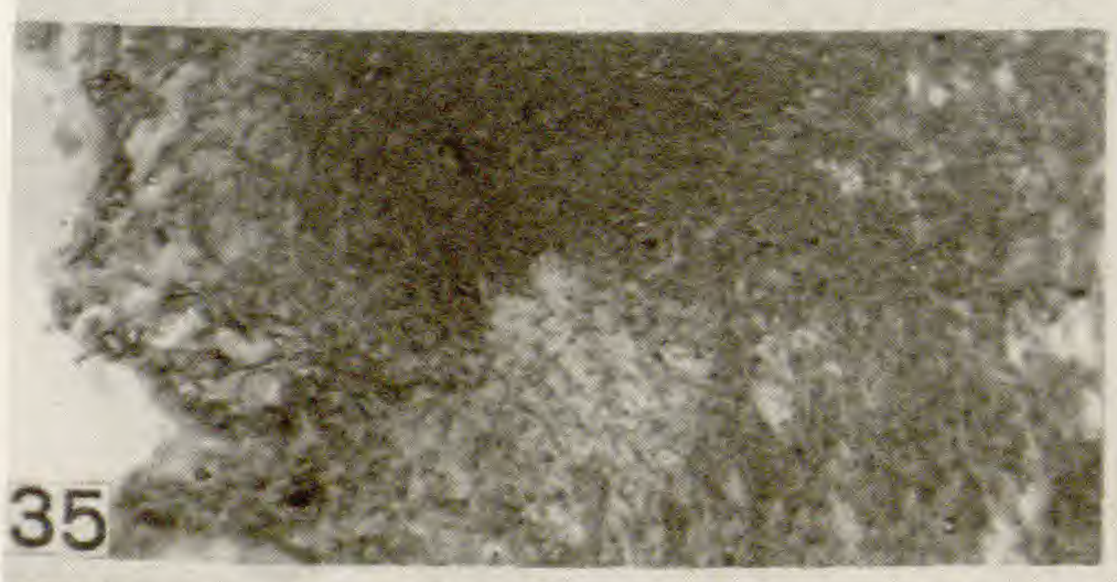
PLATE XIX

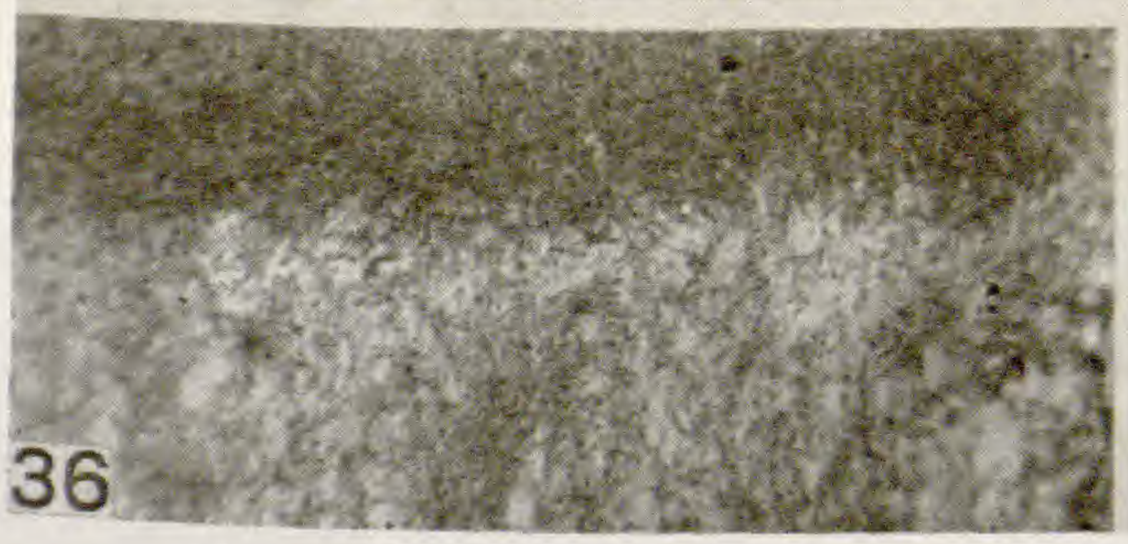












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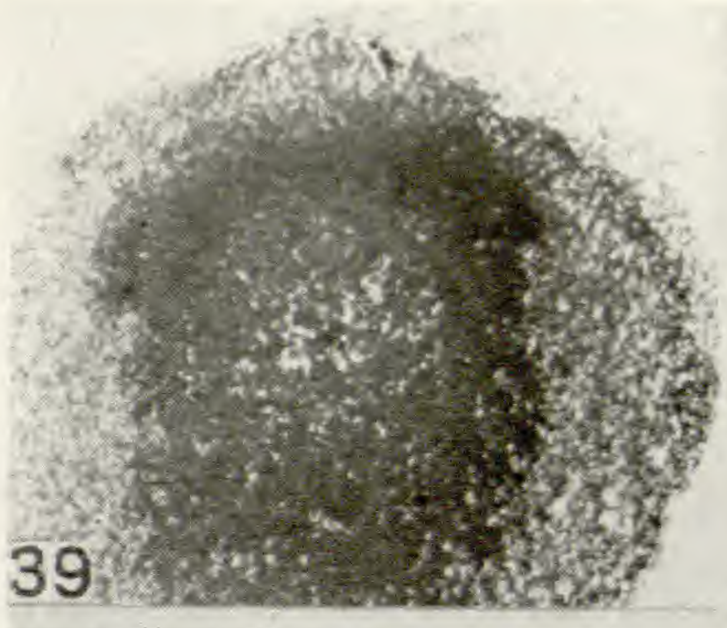
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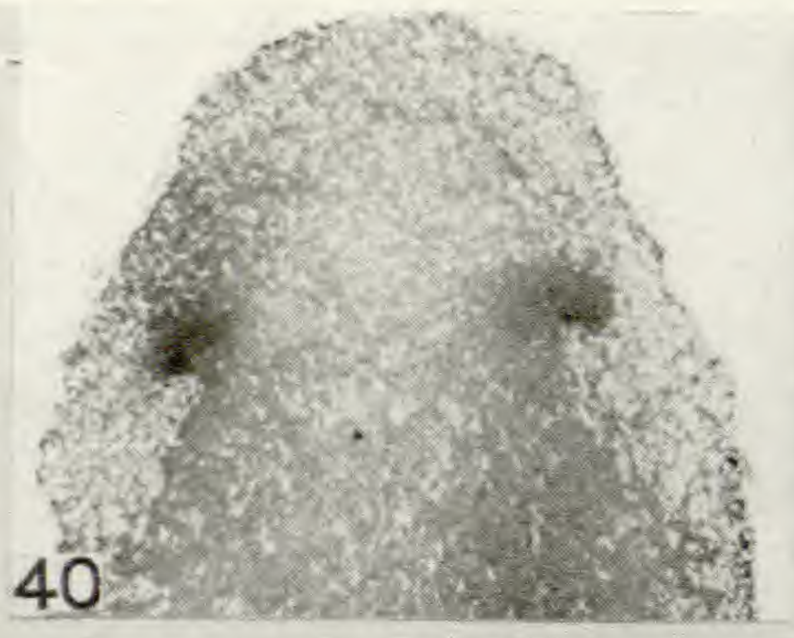
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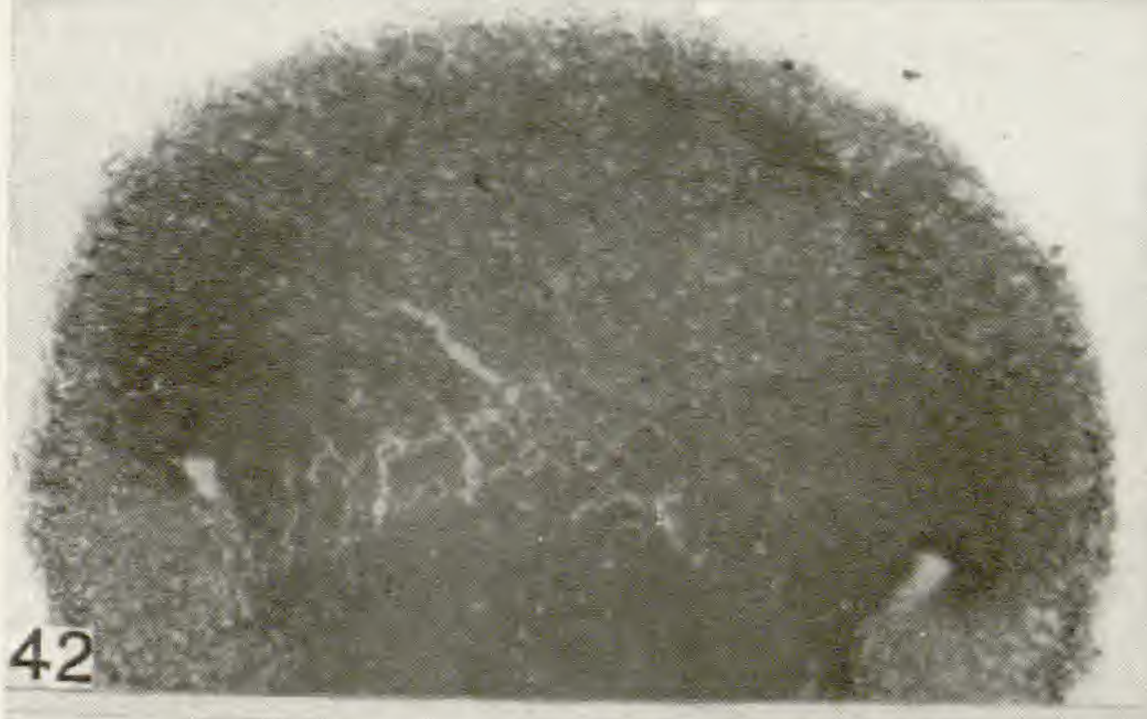
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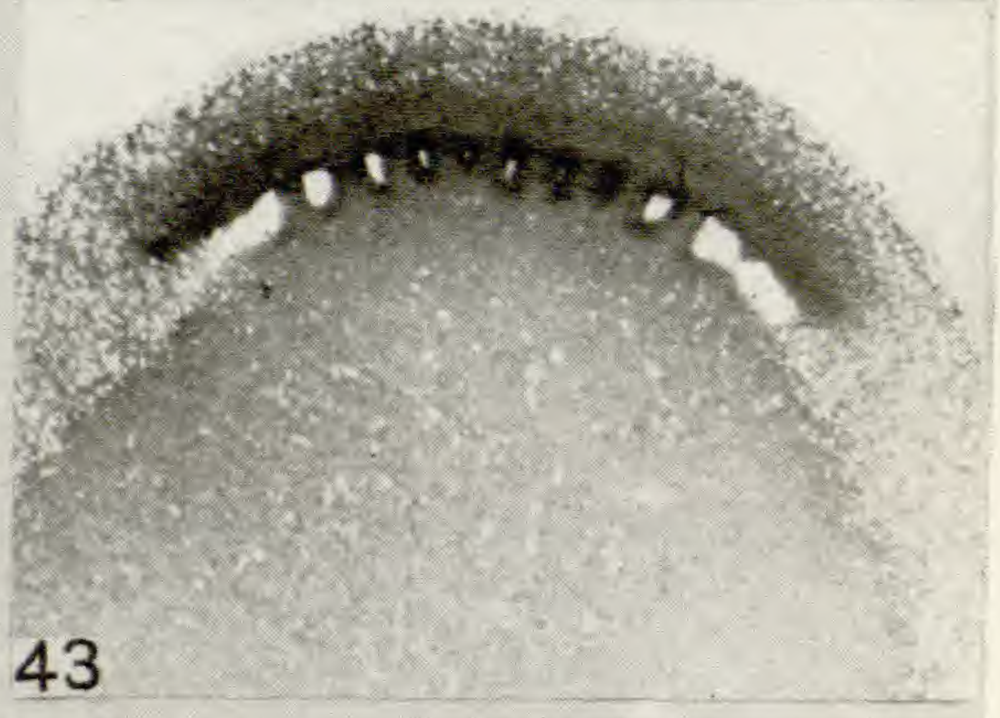
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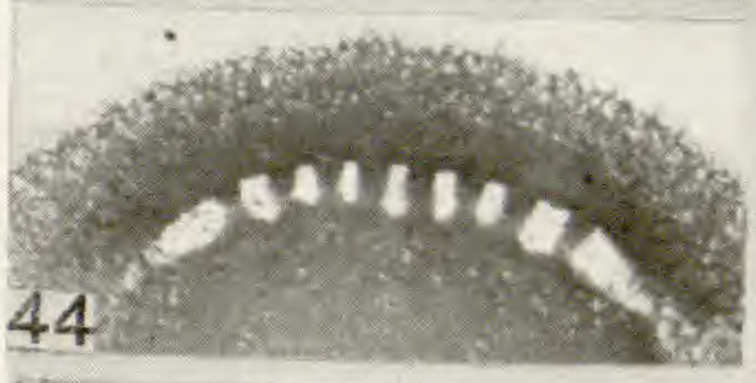
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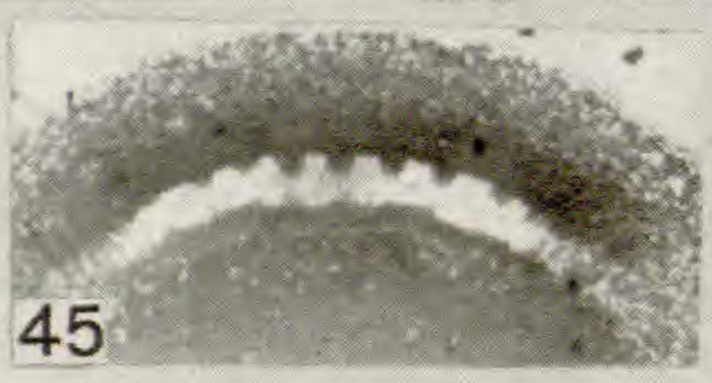
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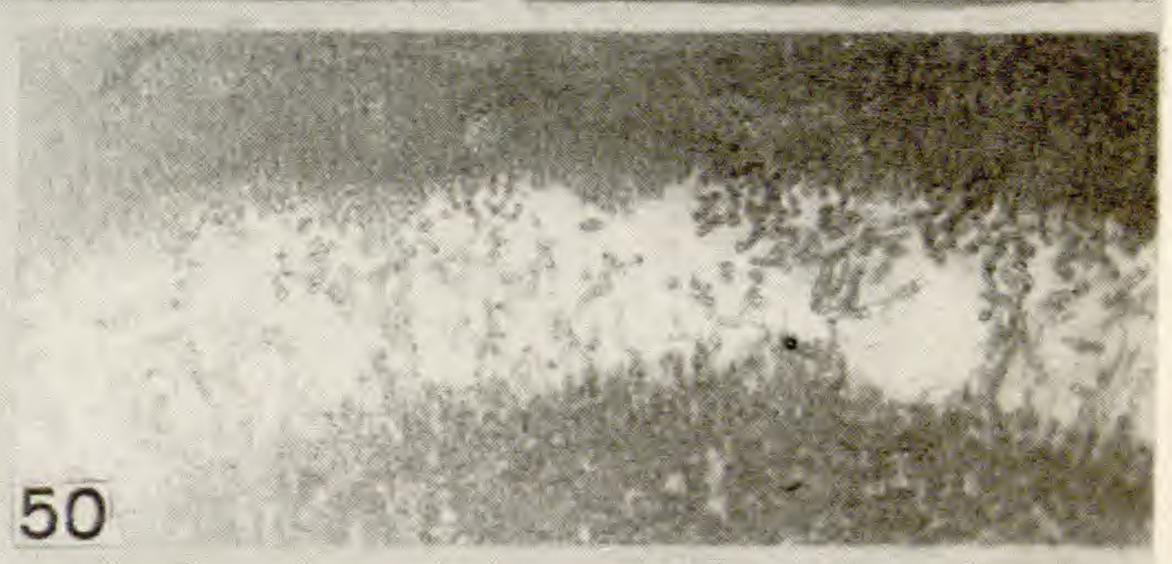
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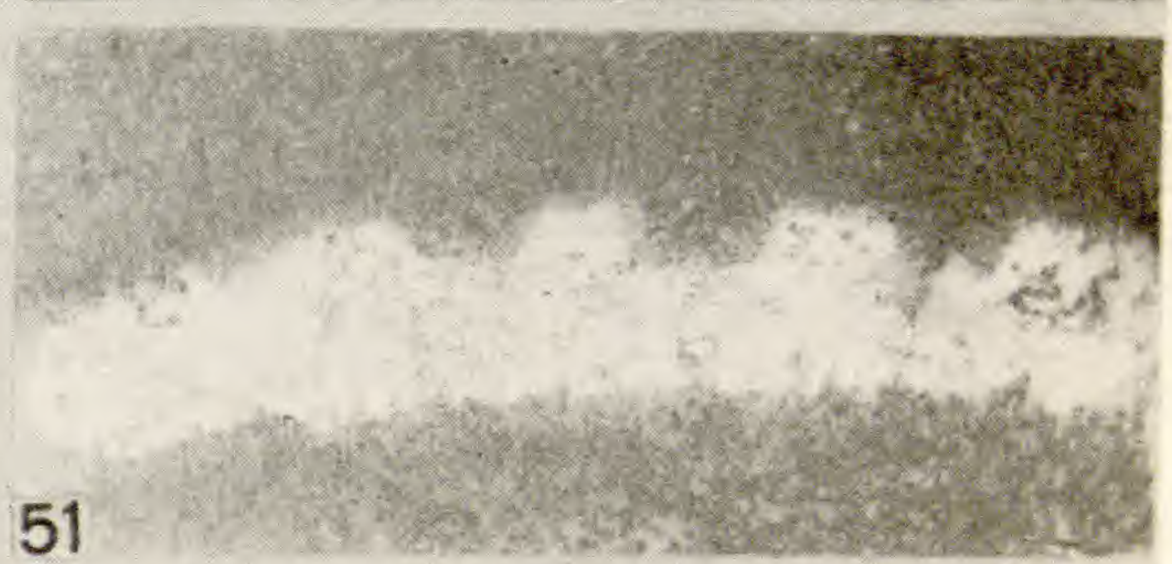
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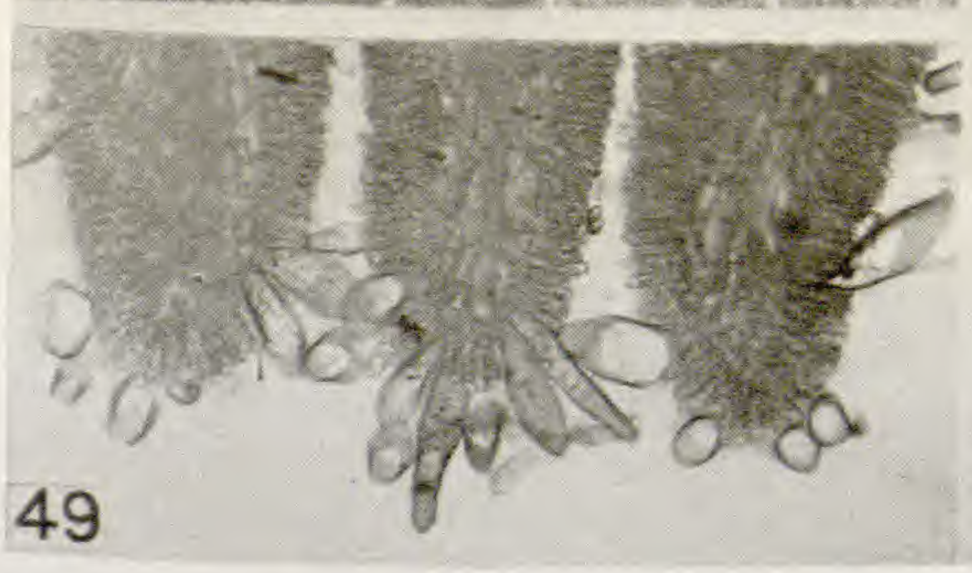
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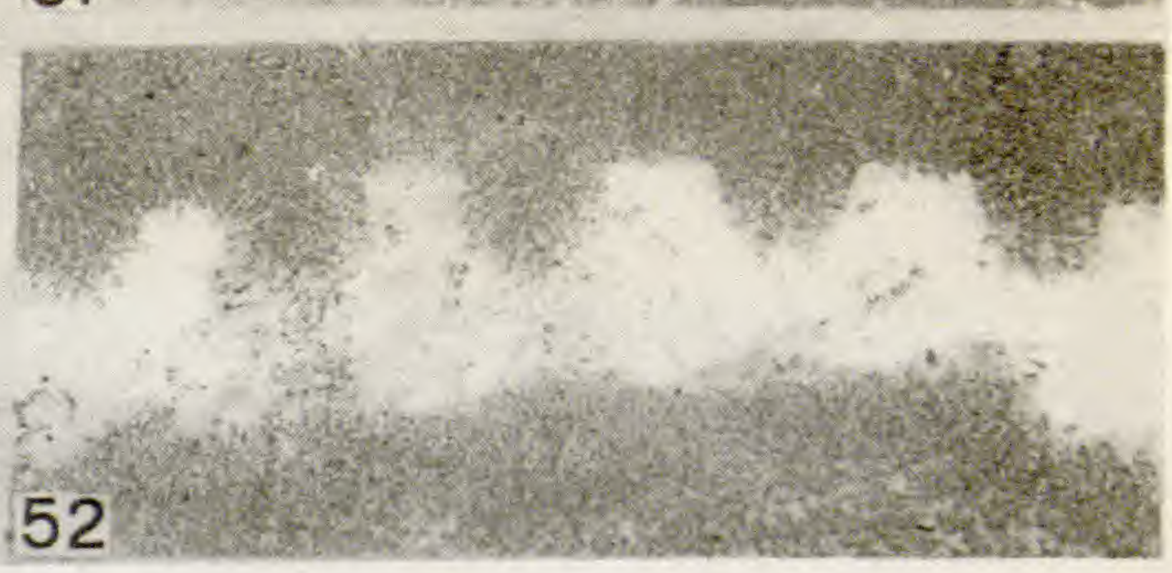
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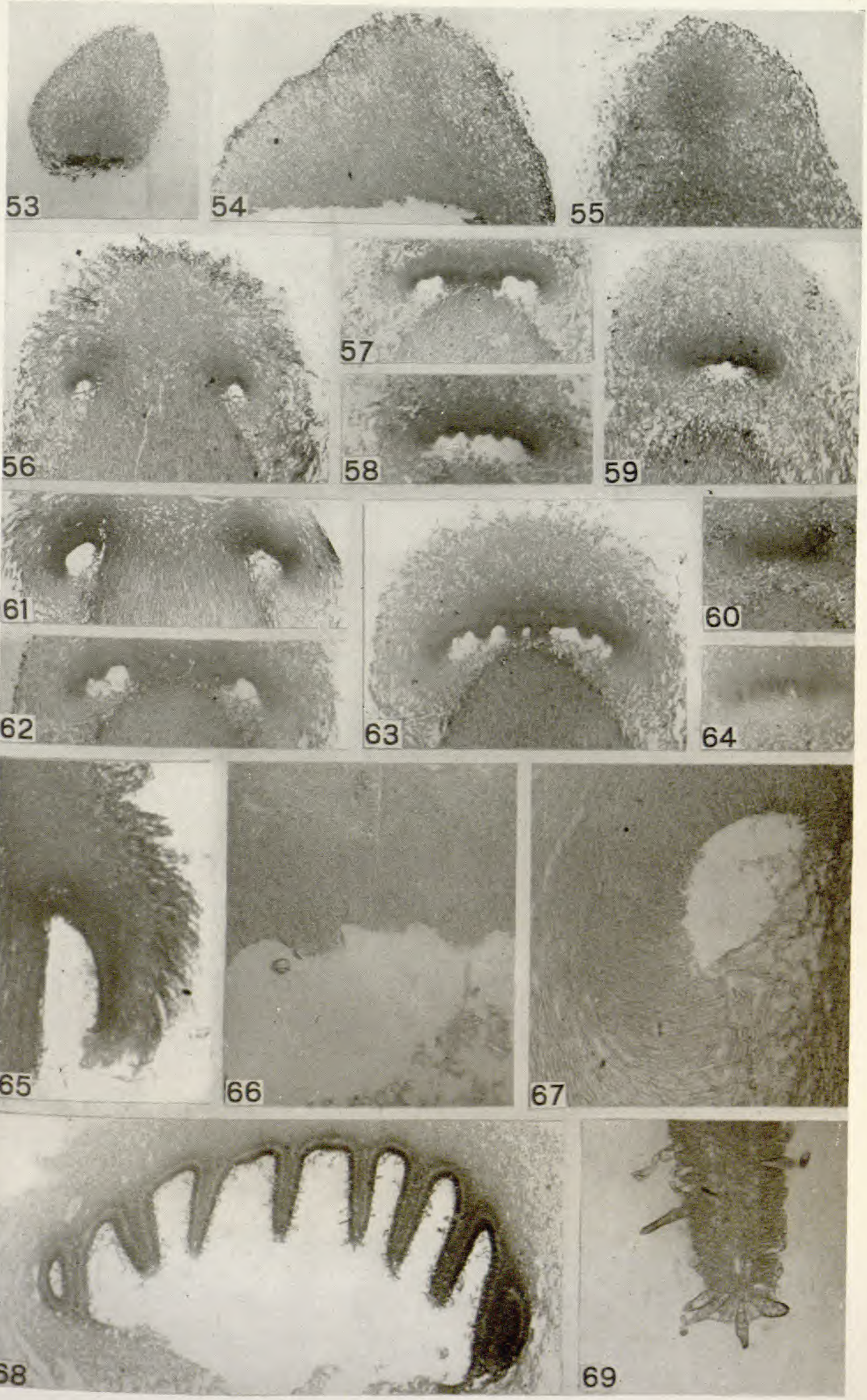

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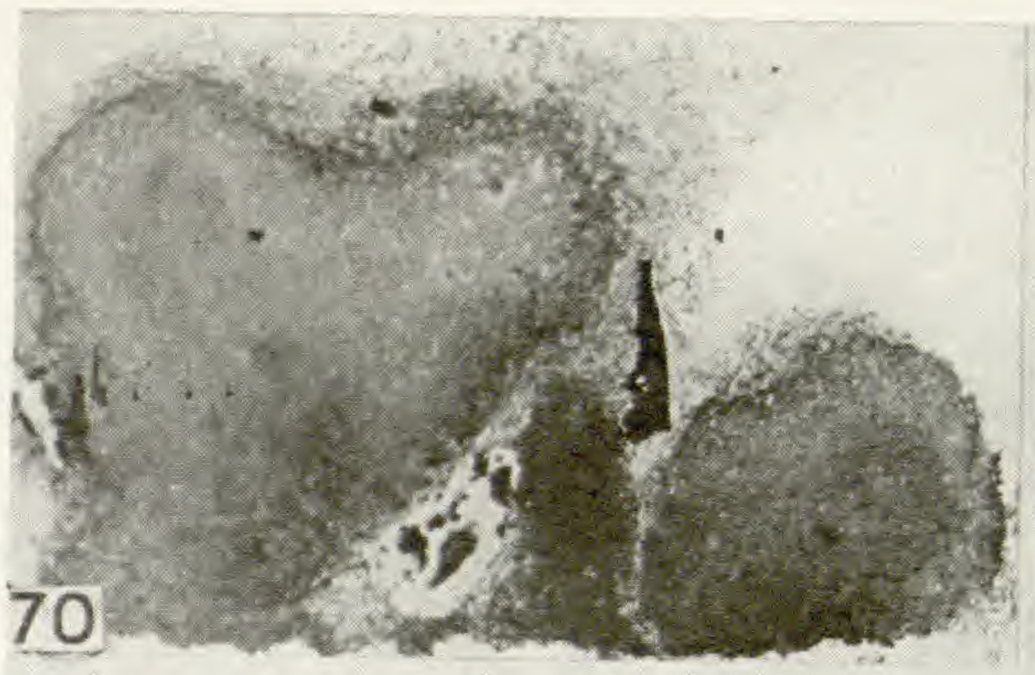
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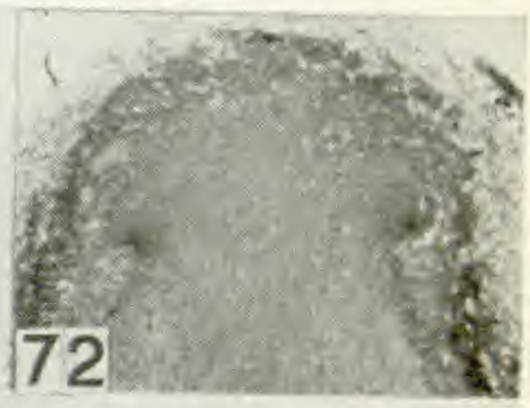
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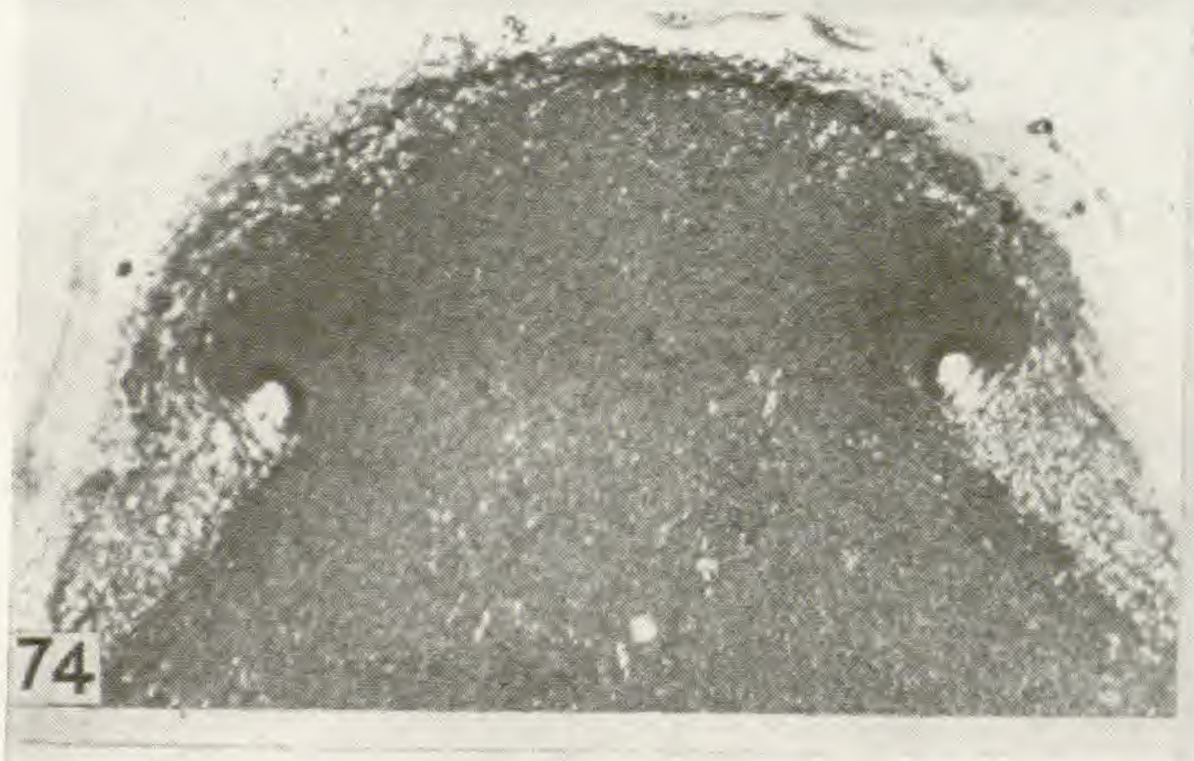
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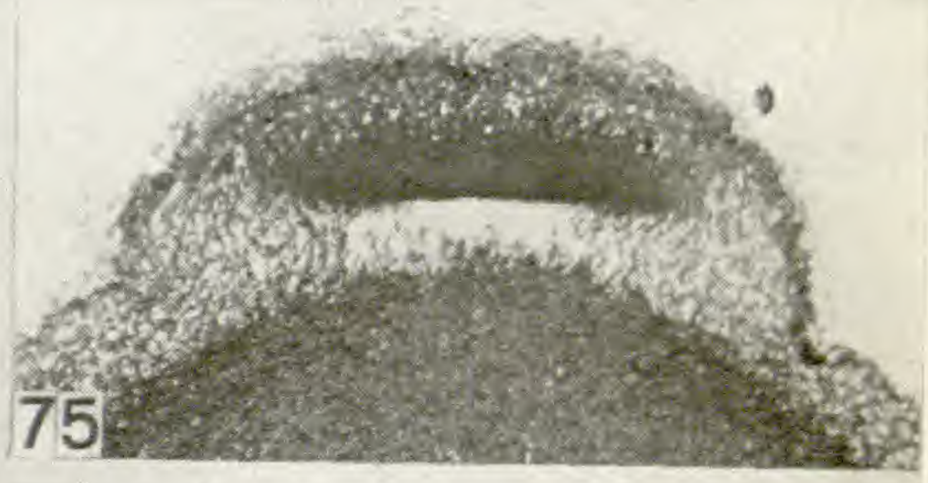
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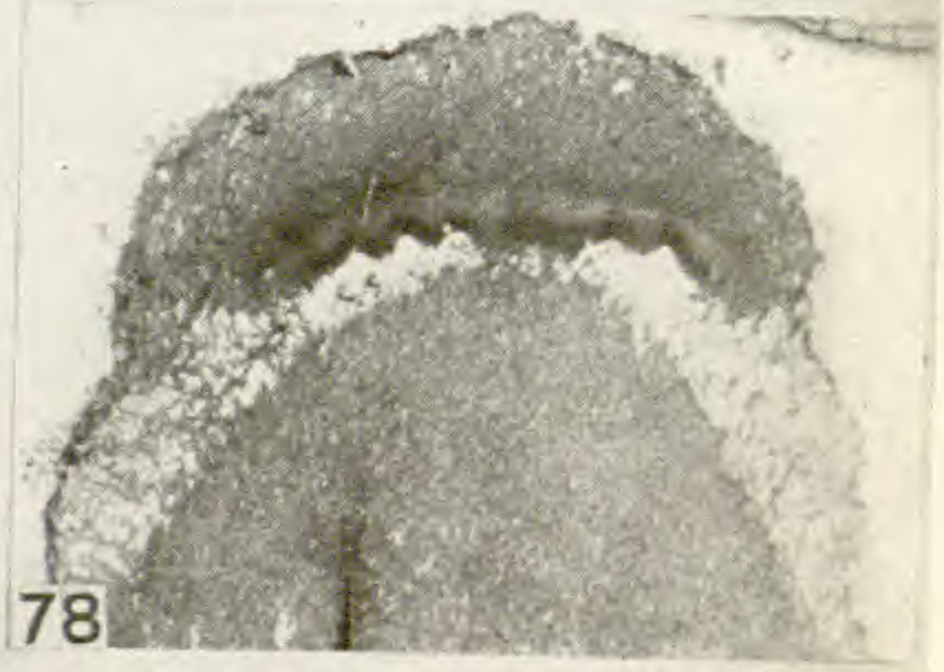
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PECTIN RELATIONS OF SCLEROTINIA CINEREA[1]

J. J. WILLAMAN

The pectic substances which constitute the middle lamellae of fleshy fruits, and thus serve as the cementing material between cells, are of great interest to the plant pathologist because of the fact that if an invading organism is to make its way into a tissue, it must either pass along the line of the middle lamella *between* the cells, or it must bore *through* the cells. In the first case a pectic enzyme at the hands of the parasite may be presupposed, and in the second case a cellulose enzyme.

The mechanism of infection by *Sclerotinia cinerea* has received particular attention recently by COOLEY (4) and by VALLEAU (8). COOLEY maintains that its hyphae in plum tissue are mainly intracellular; while VALLEAU finds them to be entirely intercellular, and produces photomicrographs to prove it. COOLEY could not demonstrate the presence of an enzyme which would dissolve the calcium pectate of the middle lamella. He also failed to find any evidence of a softening of the tissue in advance of penetration. VALLEAU, by more careful methods, noticed a marked separation of the cells in rotted tissue, and demonstrated that an extract of rotted apple would bring this about. The agent in this case is the enzyme designated by VALLEAU "pectinase," but more correctly called "pectosinase" according to the terminology of its discovery (3, 10), and of ATKINS (2). VALLEAU's illustrations show that it is secreted considerably in advance of the penetrating hyphae. The question of oxalic acid being the solvent was considered by both writers. COOLEY found appreciable amounts of the acid in rotted peaches and plums, but was not convinced that it was the middle lamella solvent. VALLEAU demonstrated that dilute solutions of oxalic acid would soften the tissues of these fruits, but not of potato, which facts convinced him that this acid is not the only factor involved in the disintegration of the middle

[1] Published with the approval of the Director as Paper no. 186, Journal Series, Minnesota Agricultural Experiment Station.

lamella. Both writers agree that *Sclerotinia* is more virulent on ripe than on green fruit, because in the ripe fruit the middle lamellae are disintegrated and allow easier penetration.

COOLEY found that the fungus coagulates soluble pectin, even when calcium is presumably absent from the medium, and that it does not dissolve precipitated "calcium pectinate" suspended in an agar nutrient medium. As to the question whether any pectic material is assimilated by this fungus, HAWKINS (**6**) found no diminution in the pentosan content of peaches which had been rotted by it, and COOLEY's observation that it could not grow on "calcium pectinate" would indicate failure to assimilate this pectic material, at least.

The present status of the question of pectin relations of *Sclerotinia cinerea*, therefore, is that it disintegrates the tissues by dissolving the middle lamella with a secreted pectosinase, and probably also with oxalic acid. What disposition is made of the dissolved pectic material, and what function the pectase serves, have not been suggested. The fungus also coagulates soluble pectin by means of pectase. Pectinase (see later for definition) has not been demonstrated.

During the course of some work on *Sclerotinia* the writer (**9**) made several interesting observations on the behavior of this fungus toward the various pectic substances. These are recorded in the following notes.

Assimilation of pectin

Before recounting the observations made on the pectic relations of this fungus it might be well to explain the nomenclature that has been adopted in this paper. It is the same as that used by ATKINS (**2**). Pectosinase is the enzyme that dissolves the middle lamella, with the formation of soluble pectin. Pectase coagulates soluble pectin to a gel in the presence of a calcium (or barium or strontium) salt. Pectinase hydrolyzes soluble pectin, and also the gel formed by pectase, to reducing sugars. Such definite names have not been given to the various pectic products; hence the particular one in question in any particular case can be told only by the context.

In order to test the assimilatory power of *Sclerotinia* toward soluble pectin, a sample of the latter was made by precipitating prune juice with alcohol, reprecipitating twice, and then drying in an oven and grinding. The resultant powder was incorporated in a mineral nutrient medium to the extent of 0.4 per cent. With no sucrose added, growth took place, but only about one-fifth as rapidly as when some sucrose was present. Whether this growth was due to the assimilation of pectin, or to sugars adsorbed during the preparation of the pectin, was not evident, since no analyses were made. To test this point quantitatively, another preparation of pectin, from peach, was made. It was decided to follow the product by analyzing for its content of pentosan. Although this is open to many objections, the chief of which is the questionable accuracy of the existing methods of pentosan determination, no other way of studying the fate of the pectin in media appeared feasible. The official (1) phloroglucide method was used. The pectin preparation showed a content of 15.0 per cent furfural, or 25.6 per cent pentosan. SCHRYVER and HAYNES (7) obtained 18.6 per cent furfural in turnip pectin. They had a far purer preparation, however, than the writer attempted to obtain.

A medium containing mineral salts and 2 per cent of pectin was prepared, and two Erlenmeyer flasks, each with 50 cc. of medium, were sterilized and inoculated with spores of the fungus. Growth was slow but typical. The medium became a gel, due to the coagulation of the pectin by the fungus. After three weeks the colony in each flask had attained a diameter of 3.5 cm. (see 9 for the writer's method of estimating the growth of this fungus). By this time also the gel had become almost completely liquefied, and the liquid portion gave no precipitate with alcohol. The colonies were carefully removed, freed from the adhering gel of the medium as well as possible, and then the two mycelia and the two media combined for analysis. The results were as follows:

Pentosan in original media	0.502 gm.
Pentosan in mycelia of culture 572	0.014 gm.
Pentosan in residual media	0.040 gm.

Thus a total of 0.054 gm. of pentosan remained in the culture flasks at the end of the period of growth, whereas 0.502 gm. was

present in the pectin at the start. Very evidently during the growth of the fungus the pectin was assimilated in part, at least. About nine-tenths of the furfural-yielding material of the pectin had disappeared. Presumably this material was utilized by the fungus for energy, but was not stored in any appreciable quantity in the hyphae. It is possible that the small amount found in the latter was due to pectic gel enmeshed in the hyphal weft.

The course of the changes involved when *Sclerotinia cinerea* grows on a pectin medium may be outlined as follows. There is first a coagulation of the soluble pectin to a gel by means of pectase, followed by a slow liquefaction of the gel during the progress of the growth of the fungus. This liquefaction is accompanied by an assimilation of at least the furfural-yielding constituents of the pectin. The fungous hyphae contain very little if any furfural-yielding bodies. It is not known whether the enzyme pectinase is involved in the liquefaction of the calcium pectate. Presumably it must be present in order to liberate soluble split products from the gel before the latter becomes available to the hyphae, although this point has not been demonstrated.

HAWKINS, on comparing the analyses of peaches before and after rotting by *Sclerotinia cinerea*, found no appreciable change in the pentosan content. This may or may not be evidence contrary to the preceding. In the whole fruit there is an abundance of carbohydrate food other than the pectin, which the fungus can utilize far more readily. As already stated, growth on the pectin medium was very slow, indicating that the fungus can utilize pectin, but with difficulty; hence it is fair to believe that in the presence of abundant sugars in a natural host the pectins are probably not drawn upon for nutrients.

Secretion of pectase

Whenever *Sclerotinia* is grown on a fruit juice medium containing soluble pectin, the latter is coagulated to a gel. This gel may vary in extent from a few suspended flocs to a solid medium, depending on the amount of pectin present. The gel is insoluble in hot water. It dissolves readily in dilute alkali and in dilute acid, and is reprecipitated from these solutions by alcohol. It is

soluble in ammonium oxalate, with the concomitant formation of crystals of calcium oxalate. In accordance with the usual nomenclature (**2, 5, 10**), therefore, the coagulum is judged to be calcium pectate, and its formation to be brought about by the enzyme pectase, secreted by the fungus. COOLEY demonstrated a similar pectic enzyme, differing from the present one, however, in that it brought about the coagulation in the supposed absence of calcium. He designated his enzyme pectinase, but that is merely a question of nomenclature. As regards the necessity for the presence of calcium, it is possible that the pectin preparation used by COOLEY contained some of this element, brought down during the alcoholic precipitation. He states that a preliminary test of the plum juice with oxalic acid showed the absence of calcium, and that this treatment was therefore abandoned as unnecessary. It is inconceivable that a fruit juice should contain no calcium whatever; hence it is very probable that the pectin coagulated by alcohol did contain some calcium, since the latter is a characteristic constituent of the alcholic precipitate of all plant juices.

In order to determine to what extent pentosans are present in the mycelium of *Sclerotiniа*, either as enmeshed pectic gel or as an actual constituent of the fungous body, a few analyses were made of felts grown on various media. The felts were removed from the media, carefully washed with water, dried between filter paper, and then placed in a desiccator over quicklime. When perfectly dry they were ground to a powder, sampled, and analyzed. The following data were obtained, expressed as percentage of pentosan in the dry mycelium:

No. 568. Grown on synthetic sucrose-salts media entirely free from pectin and pentose; sporulation abundant ..... 3.5 per cent pentosan

No. 569. Grown on whole prune juice, pectin fairly abundant; sporulation moderate ..... 1.5 per cent pentosan

No. 570. Grown on whole peach juice and apricot juice, pectin abundant; sporulation abundant ..... 0.8 per cent pentosan

No. 572. Grown on the prepared pectin medium described; sporulation absent ..... 3.9 per cent pentosan

That the figure obtained in no. 568 actually represents the pentosan content of the mycelia is doubtful. When the phloroglucin was added to the furfural-containing distillate, a bright red

color developed, which gradually changed to a reddish brown precipitate. The normal color change is a green solution at first, changing to a black precipitate. It is possible that the fungus yielded during the acid distillation some substance other than furfural which reacted with the phloroglucin. Furthermore, the media in this case contained no pentose substances whatever; hence it is very unlikely that the fungous bodies would produce more furfural-yielding materials here than in the other cases, where the possibilities of enmeshed pectin were present. The high value for no. 572 is explainable by the fact that the felt grew on a firm gel of calcium pectinate, making it difficult to obtain a clean separation of mycelium from medium in preparing the samples for analysis.

From these data it is evident that only a very small amount of pentosan is present in the felt of *Sclerotinia cinerea*, either as enmeshed pectin or as a constitutional substance. Whether the latter actually exists is not proved by the data. Furthermore, microchemical tests failed to show pectic substances in any portion of the hyphae.

A possible function of the enzyme pectase in a fluid medium, such as fruit juice, is suggested when the characteristics of the growth of *Sclerotinia* on such a medium are taken into consideration. These juices always contain soluble pectin; they also contain some calcium, but there is never any formation of calcium pectate without the presence of the fungous hyphae. As soon as the germinating spores in these media have begun to form a slight weft, this weft can be lifted from the medium with a mass of gelatinous material clinging to it. In fact, the young felt consists of a relatively small mass of hyphae imbedded in a large mass of the gelatinous coagulum. This coagulum can be removed by gentle pressure through cheesecloth in the case of young felts. As they grow older, however, the hyphae increase in bulk, become closely packed together, and the coagulum loses water and becomes firm and slippery. A mature sporulating felt consists typically of three layers: an upper white layer of loosely packed hyphae bearing the sporophores; a middle layer of black, leathery, closely packed mycelium; and a lower layer consisting of a few hyphae and the concentrated

pectin coagulum. The lower layer merges into the loose coagulum of the medium, and most of it easily sloughs off when the felt is washed in water. When an apple juice rather high in pectin is used, the whole mass becomes a rather soft gel within a few days. This gel enables the fungus to build up a semi-solid medium upon which to support aerial hyphae and sporophores.

The possible function of pectase to the fungus when invading a host tissue offers a subject for speculation, at least. The substance of the middle lamella is usually spoken of as calcium pectate. That it is the same substance as the calcium pectate gel formed by pectase is rather doubtful (**2, 5**). The work of various investigators has proved conclusively that this fungus dissolves out the middle lamella, either by the enzyme pectosinase or by oxalic acid. If it be oxalic acid, the calcium of the middle lamella would be removed as insoluble calcium oxalate, leaving a pectin residue, presumably soluble, or at least no longer capable of cementing the cell together. Whether *Sclerotinia* has the power of forming a calcium pectate gel out of such pectic residue is not known, since the nature of this material is not determined. If, however, the solution of the middle lamella be brought about by pectosinase, we have a different case to consider. We really know nothing definite concerning the action of this enzyme on the middle lamella, but presumably some simpler pectic substance would be formed along with a soluble calcium complex. The fungus would penetrate the dissolved substance of the lamella, and then reprecipitate it, presumably as the calcium pectate found in the apple juice cultures. Since it is not known whether the pectin obtained by boiling a ripe fruit in water is the same as the pectin produced by the action of pectosinase on the middle lamella, we are perhaps not entirely justified in assuming that the same substance is formed by pectase acting on dissolved middle lamella *in vivo* and on soluble pectin in an extracted fruit juice. The work of previous investigators, however, gives us good reason to believe that the pectic gels in the two cases are very similar, if not identical (**2, 5, 10**).

Given this hydrophyllic gel, what purpose may it serve the invading parasite? VALLEAU believes that the reason *Sclerotinia*

produces a firm and not a soft rot of plums is that its comparatively large hyphae completely fill the intercellular spaces produced by the collapse of the cells, and that thus the tissue retains its shape and firmness, even after rotting. The writer believes it much more probable that the laying down of considerable calcium pectate gel maintains the firmness of the rotted tissue. This view is further supported by the evidence already stated that this fungus does not consume pectic material in its metabolism to any marked degree.

Certain advantages to the fungus can be seen in this production of calcium pectate gel. Since a fruit, rotted on the tree by this fungus, typically retains its form and turgidity for some time, the fungus is enabled to sporulate (chlamydospores in this case) copiously for several days, during a period when the tree is full of ripe fruit susceptible to the rot. Again, many of these rotted fruits do not drop from the tree; they slowly shrivel up, usually without any break in the skin, and form the characteristic mummies of plum and peach orchards. The following spring these mummies imbibe water readily, due in great part to the strongly hydrophyllic calcium pectate gel, and again sporulate profusely. After this the mummies drop to the ground and remain dormant for a year, when they again imbibe water, and produce an abundance of ascospores at the time of blossoming of the fruit trees.

Summary

Sclerotinia cinerea, when grown on a fruit juice containing soluble pectin, coagulates this pectin to a gel of calcium pectate by means of the enzyme pectase. When simple sugars are available, the fungus does not assimilate pectic substances. When, however, pectin alone is available, it is slowly assimilated. The mycelium contains no pectic substances, except such as occur in particles of calcium pectate gel enmeshed by the hyphal filaments. When the fungus invades a tissue, it follows the line of the middle lamella by dissolving out the latter with the enzyme pectosinase. It probably reprecipitates the pectin of the lamella as calcium pectate. The latter, being a hydrophyllic gel, maintains the firmness of the fruit even after rotting, which is a characteristic of fruit rotted by *Sclerotinia*. This highly imbibing gel is probably also

of service to the fungus at subsequent periods by aiding the organism in acquiring a water supply. The production of pectinase is postulated but not demonstrated.

Dr. SOPHIA H. ECKERSON, of the University of Chicago, very kindly assisted the writer in the microchemical examination of the mycelia for pectin.

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CARBON MONOXIDE A RESPIRATION PRODUCT OF NEREOCYSTIS LUETKEANA

SETH C. LANGDON AND W. R. GAILEY

(WITH THREE FIGURES)

The consideration of carbon monoxide as a plant respiration product is somewhat novel from the point of view of the plant physiologist. That such may be the case seems clear from the evidence to be submitted in this paper. This investigation was carried on during the summer of 1918 at the Puget Sound Marine Station at Friday Harbor, Washington. It was expected to continue the investigation for another summer before publication. This was not possible, however, and since it now appears wholly unlikely that the work will ever be carried further by the authors, the data at hand are presented.

In previous papers by LANGDON,[1] it was shown that there was present an average of 4 per cent (by volume) of carbon monoxide in the pneumatocyst of the giant Pacific Coast kelp, *Nereocystis Luetkeana*. This statement was based on the analysis of the gas from about 1000 different specimens. The quantity of carbon monoxide varied from 1 to 12 per cent, the average being 4 per cent. The actual existence of the carbon monoxide in the gas from the kelp was demonstrated by a variety of chemical tests, by its physiological effects on animals, and by the standard spectroscopic blood tests. There was also present in the gas 15–25 per cent of oxygen, the remainder of the gas being nitrogen. There was no evidence of the presence of other gases, except, of course, water vapor, although particular search was made for carbon dioxide, hydrogen, and hydrocarbon gases.

The occurrence of carbon monoxide within a living plant at once raised the question as to its source and possible relation to anabolic or katabolic processes. In the theoretical[2] considerations

[1] Jour. Amer. Chem. Soc. **39**:149. 1917; also Puget Sound Marine Sta. Publ. 1:237. 1917.

[2] SPOEHR, H. A., Plant World **19**:1. 1916.

of the mechanisms of photosynthesis, carbon monoxide has often been considered as an intermediate step in the reduction of carbon dioxide, especially since it is so closely related chemically to formaldehyde and formic acid. Heretofore there has never been any evidence of the existence of free carbon monoxide within a living plant. The possibility of its formation by enzyme action or by decay processes was early suggested, and was the first point investigated. Finely ground kelp was allowed to undergo autolysis in contact with sea water, and the gases evolved were examined. No carbon monoxide was formed, but the gas consisted almost entirely of carbon dioxide and hydrogen.

The next step was to determine how rapidly carbon monoxide was formed within the living plant. The method of work and the further discussion will be made more clear if prefaced by a brief description of *Nereocystis*. Fig. 1 shows the kelp as it lies normally, almost submerged in the sea water, anchored to the rock bottom and supporting the streaming fronds from the top of the hollow gas-filled stipe. The plants vary greatly in size. Individuals are often 80–100 ft. in length, and contain several liters of gas which is usually at reduced pressures.[3] The inside of the gas cavity is relatively dry, and is lined with a delicate weblike structure called sieve tubes. The plant will withstand a great deal of mutilation and still continue to live and grow, if kept in sea water.[4]

It was found practicable to cut off the lower part of the stipe and in the upper part to substitute a gas of known composition for that normally present in the pneumatocyst. The cut end was closed by a cork stopper, and the plant weighted and submerged in the sea, tied to a floating support, as shown in fig. 2. After a suitable interval, changes in the gas composition were determined by analysis. In the first experiments tried, primarily to determine the rate of formation of carbon monoxide, air was substituted for the kelp gas. This was accomplished by filling the cut stipe with sea water and then emptying. This process repeated three or four times removed the small bubbles that tended to adhere to the delicate sieve tube lining of the pneumatocyst, and insured the

[3] FRYE, T. C., Puget Sound Marine Sta. Publ. 1:85. 1916.

[4] FALLIS, A., Puget Sound Marine Sta. Publ. 1:1. 1916.

complete removal of all the gas originally present. The cut end of the now air-filled stipe was corked and anchored at the surface of the sea as previously described.

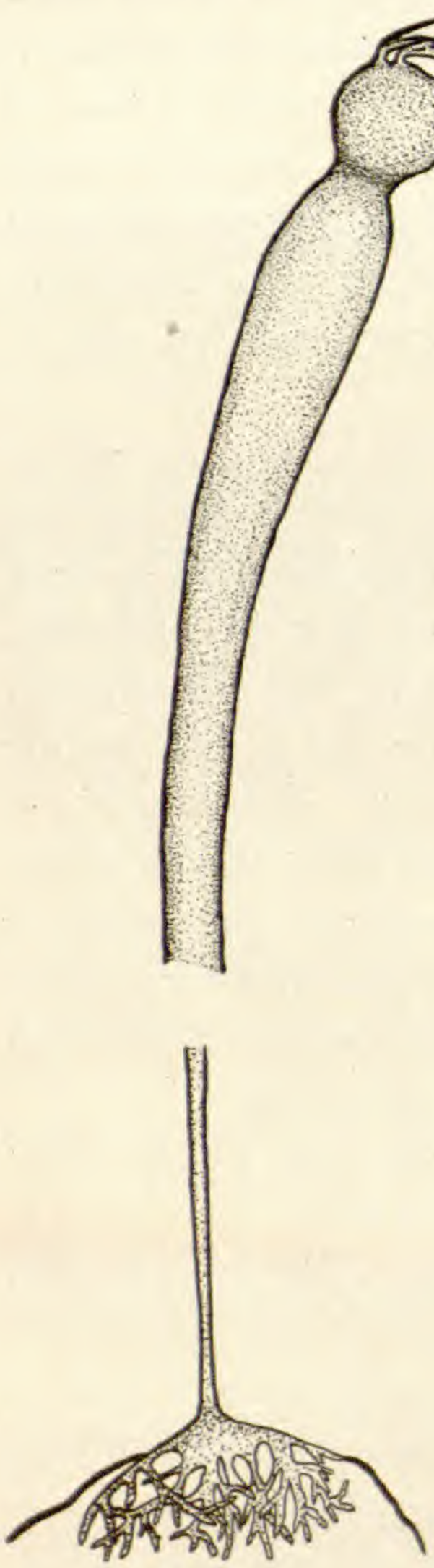

FIG. 1

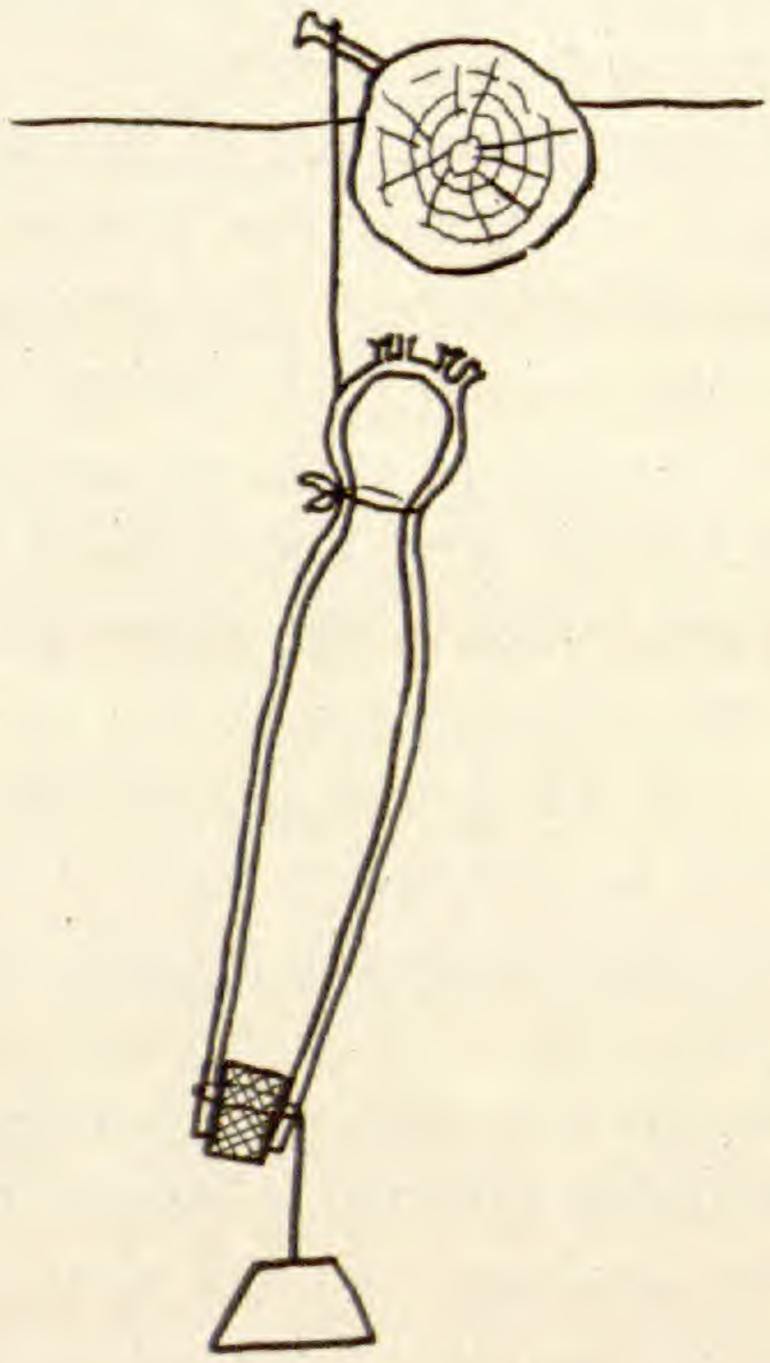

FIG. 2

Analysis of the gases from a series of these cut and air-filled plants was made after various intervals of submergence. The typical data given in table I show clearly the gradual formation of carbon monoxide, accompanied by the appearance of carbon dioxide, which latter was undoubtedly due to decay processes,

since carbon dioxide is not found in the gas normally present within the kelp. In general, the cut and corked sections of stipe remained sound enough to be tight for a week or ten days, although evidence of local decomposition was apparent. This production of carbon monoxide, when the stipe was filled with air, was confirmed by many determinations with different specimens. In most cases it appeared in quantities as great as 1 per cent or more. The presence or absence of the fronds had no relation to the carbon monoxide formation. Carbon monoxide was produced by sections cut from any part of the hollow stipe, filled with air, corked, and similarly suspended in the sea. Even the round bulblike top of the stipe,

TABLE I

Time	Percentage CO_2	Percentage CO	Percentage O_2
Start.....	0.0	0.0	20.8
24 hours.....	0.3	0.0	16.5
48 hours.....	0.0	0.4	13.0
73 hours.....	0.6	1.0	7.0
97 hours.....	1.0	3.2	6.2
110 hours.....	1.1	4.5	5.0

devoid of fronds, would form it almost as readily as if practically the whole of the plant were used.

Two other methods of displacing by air the gas originally contained within the kelp were used, but gave no difference in the final results. The first of these was to insert a rubber tube at the cut end, so that it extended the whole length of the stipe and up into the bulblike top; then to force in through the tube a large quantity of air, and thus sweep out all of the kelp gas. The other method was to draw the original gas out by connecting the cut end to a good suction filter pump. Alternately evacuating and filling with air served to accomplish the desired substitution without getting the inside of the gas cavity wet. It would be interesting to make the substitution under strictly aseptic conditions, but this the authors were not able to do.

Since carbon monoxide was formed in quantity in the living plant within a few days and was not formed by decay or autolysis, it might have been formed either as a product of respiration or as

an intermediate step in photosynthesis. If the latter, it should not appear if the experiments were carried out in the dark. To test this, boxes were constructed which were light-tight, but which would allow a ready flow of water through them. These boxes were 1 ft. square and 18 ft. long. The ends were closed by light traps, the baffle boards of which were inclined in the direction of flow of the water (fig. 3). The lids were also light-tight. All

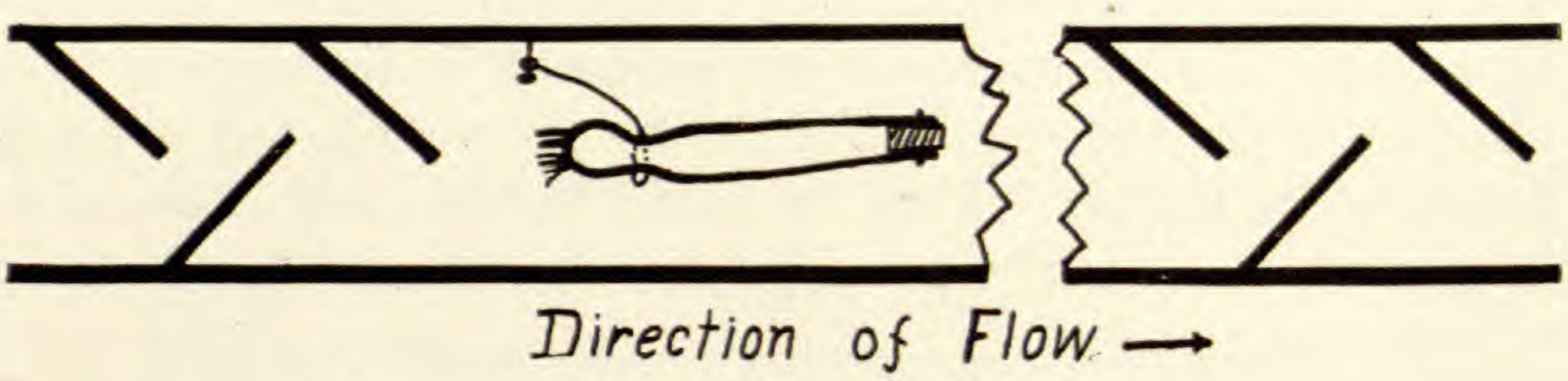

FIG. 3

holes and cracks were closed with pitch, and the whole interior painted a dead black. The boxes were weighted so as to just float; the waves washed entirely over them except when the water was perfectly calm. The boxes were anchored in the bay (Friday Harbor, Washington) where the tidal currents are heavy, so that there was practically always a flow of water through the boxes. The boxes were large enough to hold several specimens without impeding the flow of water.

In the first experiments, the top 18 inches of stipe from 10 kelps were filled with air by displacement with sea water and corked and placed in the dark boxes. Half of them had the fronds removed. After 5 days in the dark they were removed and the gas analyzed. All showed carbon monoxide. The range was from 0.4 to 1.7 per cent, with an average of 0.7 per cent. The 20.9 per cent of oxygen in the air with which they had been filled had practically disappeared, and there was about 4 per cent of carbon dioxide. The oxygen was used by respiration and decay processes. These data were checked by extended experiments, and it was made certain that in the dark as well as in the light carbon monoxide was formed regularly in the air-filled section of the stipe, and that there was no relation between its appearance and the presence or absence of the fronds.

The appearance of CO_2 and the lowering of the oxygen content when the unmutilated plants were kept in the dark for some time were shown by the following experiments. Twelve whole plants were gathered from the same bed, precaution being taken to avoid in any way disrupting the gas cavity. The gas from 6 of them was analyzed at once and showed an average of 15 per cent of oxygen, 3.2 per cent of carbon monoxide, and no carbon dioxide. The other 6 plants were placed intact in the dark boxes, and after being anchored out in the tidal currents for 6 days showed the following average gas composition: oxygen 4.7 per cent, carbon monoxide 2.9 per cent, and carbon dioxide 0.5 per cent. There was therefore a marked decrease in the oxygen content, and an appearance of carbon dioxide which is practically never present in the kelp when freshly collected, while there was practically the same carbon monoxide content.

The substitution of gases other than air for those normally present was next undertaken. As a result of more than 40 carefully made experiments, in which nitrogen was substituted for the kelp gas, it can be stated confidently that no carbon monoxide was formed, either in the light or in the dark, either when fronds were present or when they had been removed, or at any intermediate time between the initial filling and the 8–10 days before decay had become so pronounced that observations could no longer be made. It should be remarked that a small percentage of carbon dioxide was generally formed, even though there was no oxygen present.

The nitrogen was prepared in three ways. One method was by heating a mixture of concentrated aqueous solution of sodium nitrite and ammonium chloride. The products other than nitrogen from this reaction are sodium chloride and water. The first few liters of gas evolved were discarded and the remainder showed no oxygen. The gas was washed through strong sodium hydroxide solution. The second method of preparing "nitrogen" was to absorb the oxygen from the air by means of alkaline pyrogallol. That the failure of the plant to produce carbon monoxide was not due to a trace of some unknown impurity, introduced into the nitrogen as chemically prepared and purified, was made certain by the use of nitrogen obtained by the fractional distillation of liquid

air. This nitrogen procured from the Linde Air Products Company contained a trace of oxygen, about 6–8 parts per thousand by volume, but the results obtained with it were the same as with the nitrogen prepared in other ways.

Similar experiments were carried out in which hydrogen was substituted for the kelp gas. The 15 determinations made showed no formation of carbon monoxide within 5–7 days, either in the light or in the dark. Here, as in the case of the nitrogen-filled kelp, a small percentage (1–9 per cent) of carbon dioxide was formed. It should be remarked that there was always a marked reduction in pressure for hydrogen-filled kelp. This amounts to an absorption or diffusion out of hydrogen. The whole relation of hydrogen in this connection deserves more exhaustive study.

The hydrogen used was from two sources: (1) the action of dilute sulphuric acid on the so-called arsenic free zinc; (2) a commercial product prepared by electrolysis.

A number of sections of kelp stipe were filled with a mixture of nitrogen and oxygen, both chemically prepared. The initial composition was 15.2 per cent oxygen and the remainder nitrogen. After 6 days' exposure carbon monoxide had been formed in all cases, the quantities ranging from 0.8 to 2.1 per cent. The oxygen content decreased and some carbon dioxide was formed just as in the case of the specimens filled with air. Kelp filled with a mixture containing 26.2 per cent of oxygen and the rest hydrogen showed in the same time a similar formation of carbon monoxide and a corresponding decrease in oxygen accompanied by the appearance of carbon dioxide.

The evidence so far presented seems to point to the inevitable conclusion that the carbon monoxide is a product of respiration, since it is not formed by decay or autolysis, and is formed only when there is oxygen present in the pneumatocyst.[5]

A similar supplementary series of experiments was made which supported the conclusion that the carbon monoxide was formed by a katabolic process. The gas-filled corked sections of

[5] That the CO was a respiration product was early suggested by RIGG. This conclusion was based on a special knowledge of the physiology of the plant. It was his belief that the effect was intimately related to the sieve tubes.

stipe were not put back in their normal habitat, sea water, but were left out in the air. It should be recalled that this plant will withstand a very considerable amount of desiccation and will resume growth when returned to the sea. Sections of stipe filled with air and placed in a warm, dry, dimly lighted attic developed 0.7–4.7 per cent of carbon monoxide in 5 days. The oxygen content lowered to about 4 per cent and there appeared 1–2 per cent of carbon dioxide. Similar results were obtained when specimens of air-filled kelp were placed in good daylight (not direct sunlight) or when kept exposed to the air in a dark room. Kelp filled with nitrogen or with hydrogen and allowed to stand (dry) in the air developed no carbon monoxide. On the other hand, under the same conditions, but filled with mixtures of oxygen and hydrogen, or oxygen and nitrogen, carbon monoxide was produced within a few days.

That carbon monoxide was not formed in dead kelp was shown in the following manner. A number of plants were killed by being placed for 10 minutes in sea water which was maintained at a temperature of 50° C. The stipes, full of air, were corked. Some of them were placed in the sea water in the light and some anchored out in the dark boxes. Another set similarly treated was placed in air, some of them in the light and the remainder in the dark. Analysis of the gas from the various specimens 6 days later showed no carbon monoxide. There had been a slight decrease in the oxygen content and the formation of 2 or 3 per cent of carbon dioxide. Exactly similar results were obtained when the kelp was killed by being placed in N/50 Cu SO_4 for 18 hours.

A series of experiments was started to determine what would happen if kelp were filled with oxygen-free nitrogen or hydrogen to which had been added a small quantity of carbon monoxide. These experiments were not completed, but it became clear that the change, if any, was slight.

Nereocystis Luetkeana seems to be remarkably well adapted to research on gas exchange of living cells. By the use of the very refined methods of gas analysis which are now available, some very interesting and valuable information might be gained. It is possible that traces of hydrogen or carbon monoxide not revealed

by the technical gas analytical methods used in this work may be playing important rôles in plant processes.

The gas contained in the hollow cavities of several other growing plants was investigated, but in no case was carbon monoxide found. The gas obtained from a vigorously growing garden pumpkin contained 18 per cent of oxygen with no carbon dioxide or carbon monoxide. Very similar results were obtained from the gas contained in the hollow stems of a species of *Equisetum*, the hollow stems of a species of *Petasites* (colt's foot), from the pods of green garden peas, and from the seed pod of the soft maple. In the original paper it was reported that there was no carbon monoxide in the gas obtained from the vesicles of *Egregia Menziesii*, or in that obtained from *Fucus evanescens*.

The Scripps Institute collected gas for the author from two of the southern algae. One of these, *Macrocystis pyrifera*, showed no carbon monoxide; while the other, *Pelagophycus Porra*, showed a small carbon monoxide content. These gases were collected in quart fruit jars and shipped from La Jolla, California, to Seattle, Washington, for analysis. To be definitely certain of the presence of carbon monoxide in the gas from the elk kelp, *Pelagophycus Porra*, it would be necessary to examine the freshly collected gas. It is interesting to note that *Pelagophycus Porra* is very closely related to *Nereocystis Luetkeana*, and is very similar in structure, the inside of the gas cavity of both being characterized by the presence of a pith web.

The occurrence of free carbon monoxide within a living plant is unique, so far as the authors have been able to ascertain. Its further study in relation to plant life should prove interesting, and it is not without a measure of regret that the authors leave this field to other investigators.

Summary

1. The existence of a percentage of carbon monoxide in the gas contained in the pneumatocyst of the Pacific Coast kelp *Nereocystis Luetkeana* is confirmed.

2. The substance of the kelp when ground and allowed to undergo autolysis and decay does not form carbon monoxide by enzyme action or fermentation process.

3. Kelp plants, in which the gas normally present within the floater is replaced by air, form several per cent of carbon monoxide within a few days.

4. The formation of carbon monoxide takes place only when oxygen is present as one of the gases within the floater. No carbon monoxide is formed when the floater is filled with nitrogen or hydrogen.

5. Light does not affect the rate of formation of carbon monoxide.

6. The gas obtained from the cavities of various other plants failed to show a similar occurrence of free carbon monoxide.

7. The percentage of free carbon monoxide which occurs in the floater of *Nereocystis Luetkeana* is considered to be a respiration product for the following reasons. It forms only when oxygen is present within the floater; it forms as readily in the dark as in the light; it is not formed by enzyme or fermentation process when the substance of the plant undergoes autolysis and decay; and it is not formed in killed plants.

In conclusion, the authors wish to acknowledge the many courtesies extended to them by Dr. T. C. FRYE, Director of the Puget Sound Marine Station.

EVANSTON, ILL.

BRIEFER ARTICLES

JAMES M. MACOUN

(WITH PORTRAIT)

JAMES M. MACOUN died on January 8, 1920, in Ottawa, Canada. During the previous summer, while exploring Jasper Park and adjoining territory in British Columbia, he was taken ill, and on his return to Ottawa in the fall his condition gradually became worse, until the end came. Being an excellent man and an accomplished naturalist his death will be mourned far beyond the confines of this continent, for

Mr. MACOUN was an exceptionally active man, who kept in constant touch with learned men and institutions nearly all over the world. His career covers a great field. He was born in Brockville in 1862, and when his father, Professor JOHN MACOUN, took charge of the botanical and zoölogical work of the Geological Survey, JAMES MACOUN became his assistant in 1883.

From the time he entered the service, MACOUN specialized in botany, and in addition to other duties assisted Professor JOHN MACOUN in the preparation and publication of over 1200 pages of botanical work, and two editions of an annotated list of the birds of the Dominion. He was appointed Assistant Naturalist in 1898, and Botanist in 1917. Since 1911, when his father moved to British Columbia, much greater responsibility was thrown on him. In 1918, because of his wide knowledge in the different branches of natural history, he was appointed Chief of the

Biological Division to the Geological Survey, and was looking forward to a wider field of public service.

MACOUN's great ability to do work of a special nature satisfactorily was early recognized in the Geological Survey, and in 1891, when it became necessary to investigate the fur seal fisheries of the Pacific Islands on behalf of Great Britain and Canada, he was chosen by Dr. G. M. DAWSON, then Director of the Geological Survey and Bering Sea Commissioner for Canada, to go with him. His services in the study of the habits and life history of the fur seal proved so valuable that he was retained on this special work in 1892 and 1893, and was sent to Europe as an expert in connection with the fur seal arbitration. In 1896 he was again sent to Bering Sea, and again in 1914. In 1911 he spent 10 weeks in Washington as one of Canada's representatives at the fur seal conference. Because of this special international work he was very highly commended by Lord BRYCE, then British Ambassador at Washington, and received a C.M.G. for his services.

The field work of the staff of the Geological Survey takes the members to many parts of Canada, and mainly to the outlying, or least civilized parts of the Dominion, and during the 36 years of service MACOUN had his full share, enduring in some of the expeditions very severe hardships. As an example it will be remembered that in 1910, while studying the flora and fauna of the west coast of Hudson Bay, the ship which carried him and his party was wrecked, and they had to attempt the return to civilization in a small boat; but fortunately they were rescued and taken to Fort Churchill, from which point the party made the overland trip to Lake Winnipeg on foot, in the depth of winter, reaching the telegraph line after having been almost given up for lost.

As an evidence of the splendid work done by himself and his father, there are now in the possession of the National Herbarium of the Geological Survey, over 100,000 specimens of the flora of Canada. In addition, both men may be named among the founders of the Royal Victoria Museum of Canada, and perhaps half of the bird specimens, numbering about 14,000 in all, were supplied by the MACOUNS.

As a botanist, MACOUN proved himself a keen observer, and the enormous collections which he brought home from his expeditions contain a most excellent foundation for the knowledge of the Canadian flora. He discovered many new species, and his talent to select and prepare specimens so as to represent the variation of a number of plants was unsurpassed. Although he was more familar with the Canadian flora than any other botanist, he contributed but very little in print.

It was always his hope to be able to work up his collections sometime, but not until he had managed to acquire the literature necessary. The botanical library at Ottawa was not sufficiently provided with books in his line of work, and he was too conscientious to publish for the mere sake of publishing. It was a matter of small importance to MACOUN to make new species, he rather disliked it; he took more interest in contributing to the geographical distribution of plants, and his collections in this respect are, to say the least, invaluable. He took great interest in the difficult genus *Carex*, and he knew all the Canadian species at almost any stage of development, indeed all the varieties and forms. We owe to MACOUN the rediscovery of *Carex Franklini* and *C. petricosa*. As a botanical correspondent MACOUN was indefatigable, and one must remember that as Chief of the Biological Division he had to attend to a great amount of routine work.

In private life MACOUN took a deep interest in all questions bearing upon the progress of humanity. He took a keen and active interest in labor and sociological questions, and of late was specially interested in the returned soldier problem. MACOUN made and left many friends, and not the fewest of those who will miss him most are among the great class known as "labor." His death is a severe loss to natural science, and wherever records are given of the progress of natural science in Canada his name will be remembered, for he was one of its founders. His simple mode of life well corresponded with his sincerity as a friend, and his never failing sympathy for the poor.—THEO. HOLM, *Clinton, Md.*

CURRENT LITERATURE

NOTES FOR STUDENTS

Light and growth.—BAKHUIJZEN[1] gives a theoretical discussion of the possibility of photo-growth response as the basis of phototropic reaction. He accepts BLAAUW'S[2] view that the effect of light on the longitudinal growth is the basis of phototropic response, which is the resultant of the effect of unilateral light on the growth of the two flanks of the plant organ. This is DE CONDALLIS' old theory of phototropic response. He calculates VOGT'S data on the *Avena* coleoptile, which he believes confirms this view. From this work he believes that the retarding effect of a given dosage of light accounts for the phototropic response, and that the later accelerating effect is not involved. He finds that the effect of omnilateral fore-illumination on later phototropic response to unilateral illumination can be explained by the joint effect of the two kinds of illumination upon the growth of the two flanks, and that there is no necessity of assuming that omnilateral illumination changes the sensitiveness of the organ. This agrees more nearly with ARISZ' interpretation of his results, and is quite opposite to the view of BREMEKAMP. CLARK'S results, in which he found that omnilateral fore-illumination increased the sensitiveness of the organ in negative phototropic response, can be similarly explained. The time during which the light is applied, as well as the total energy, is important, and this will explain the results obtained by unilateral illuminations followed by omnilateral illuminations on the photo-growth basis.

LAURENS and HOOKER,[3] working on *Volvox* with various ray lengths of equal energy value, find that wave length λ 494 $\mu\mu$ has the highest stimulative value, and that the efficiency decreases with both shorter and longer wave lengths. The measurements were made both on the basis of relative duration of the presentation time and the relative rate of locomotion (and precision of orientation). Other authors have found the optimum for photo-perception and photo-growth response for various forms in this general region of the spectrum.

GUTTENBURG[4] has shown by several methods that in the coleoptile of *Avena sativa* the total plane of unilateral illumination is quite as important in

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[1] BAKHUIJZEN, H. L., VAN DE SANDE, Photo-growth reaction and disposition to light in *Avena sativa*. Konin. Akad. Weten. Amster. **22**:1–16. 1919.

[2] BOT. GAZ. **59**:67–68. 1915.

[3] LAURENS, H., and HOOKER, H. D., JR., Studies on the relative physiological value of spectral light. II. The sensibility of *Volvox* to wave-lengths of equal energy content. Jour. Exp. Zool. **30**:345–368. 1920.

[4] GUTTENBURG, H. V., Untersuchungen über den Phototropismus der Pflanzen. I. Über die Abhängigkeit der phototropen Erscheinungen von der Grösse der Beleuchteten Flache. Ber. Bot. Gesells. **37**:299–304. 1919.

determining photo-presentation as the light intensity or the duration of light exposure.

GUTTENBURG[5] has also worked on the question of whether it is the direction of the ray or the relative intensity of the light on the two flanks of the plant organ that determines the phototropic orientation. His evidence points to the former as the determining factor. It is doubtful, however, whether his methods are to be compared in reliability with those of MAST,[6] which seem to prove the intensity theory.

SCHANZ[7] has just published a striking article on the effect of light of different ray lengths on the development of plants. This is one of the very rare articles that makes a really large contribution to the subject. This is assuming, of course, that his results and conclusions will be confirmed by later work. The plants were grown in beds covered with glass of various kinds that cut out different regions of the spectrum. The nine beds received the following light: (I) without cover, ray lengths λ 300 $\mu\mu$ and longer; (II) covered with ordinary window glass, ray lengths λ 320 $\mu\mu$ and longer; (III) euphos (*a*) glass only, ray lengths λ 380 $\mu\mu$ and longer; (IV) euphos (*b*) glass only, ray lengths λ 420 $\mu\mu$ and longer; (V) red glass only, ray lengths λ 560 $\mu\mu$ and longer. By combining yellow, green, and blue violet glass with euphos glass he got yellow, green, and blue violet lights respectively that were free from most of the ultra violet rays. He numbered the yellow VI, the green VII, and the blue violet VIII.

Cucumbers grown in these beds showed a rather rapid increase in rate of growth and vigor as one passed from bed I to bed V; that is, as more and more of the ultra violet and other short rays were removed. From bed V to VIII the rate of growth and vigor fell off. The curve representing the growth rate in the various beds was a mathematical curve with the peak at V. *Petunia*, *Fuchsia*, *Chrysanthemum*, *Lobelia*, *Begonia*, and *Oxalis esculenta* showed similar curves. In fact, practically all of the plants studied showed similar behavior in the rising part of the curve (beds I to V), but several showed irregularities in the falling part of the curve (beds VI to VIII). The potato was weakest in yellow, stronger in green, and still stronger in blue violet. In green lettuce the leaves became continuously longer and more slender as one passed from beds I to V. They were very slender in red, and there was a misproportion between midrib and flat portion of the leaf. In yellow the disturbance was still greater and the plants showed poor chlorophyll development. In green these plants showed similar but less marked dis-

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[5] GUTTENBURG, H. V., Untersuchungen über den Phototropismus der Pflanzen. II. Neue Versuche zur Frage nach der Art der Lichtperception. Ber. Bot. Gesells. **37**:304–310. 1919.

[6] BOT. GAZ. **51**:304–305. 1911.

[7] SCHANZ, FRITZ, Wirkungen des Lichts verschiedener Wellenlänge auf die Pflanzen. Ber. Bot. Gesells. **37**:430–442. 1919.

turbances. In blue violet they were more vigorous and deep green. In short, the green lettuce showed a deficiency in chlorophyll development in yellow and green lights. Some other irregularities appeared in certain other plants.

The blooming was earlier (green lettuce, *Fuchsia*, beans, tomatoes) as one passed from bed I to IV; that is, the euphos glass, which cut out much of the ultra violet rays, favored early blooming to a marked degree. The number of fruits increased from bed I to IV. In red, yellow, green, and blue violet the blooming was deferred and the number of fruits reduced.

The ultra violet rays have a very important relation to anthocyanin development in the epidermal layer. In red-leaved lettuce the red color fell as one passed from bed I to bed III where no red developed. No anthocyanin developed in beds IV to VIII. *Celosia Thomsoni*, red-leaved begonia, and red-leaved beet acted similarly, except that anthocyanin developed in the midribs and petioles of the last in all the beds. When any of these plants were grown in beds where anthocyanin did not develop, and were then transferred to bed I, anthocyanin began to appear in 2 days and was fully developed in 8 days. When plants grown in bed I and bearing anthocyanin were transferred to beds III to VIII, the new leaves unfolding in the latter beds were without anthocyanin.

In all plants except one SCHANZ found no evidence that the red pigment functioned in protecting the plant against the injurious action of the ultra violet of the solar spectrum; for when plants were transferred from bed IV, where no red pigment developed, to bed I, no injury appeared, but the leaves soon developed the pigment. Red beech was the exception. When this plant was transferred from bed IV to bed I, the old leaves without pigment died within a few days, and the new leaves unfolding developed the red pigment.

The rate and percentage of germination of seeds (lettuce and stinging nettle) increased from bed I to bed IV. Repeated cultures on these forms showed that ultra violet interferes with germination.

When etiolated seedlings (bush beans, soy beans, potatoes) were transferred to the beds, the order of greening, beginning with the fastest, was red, euphos *b*, euphos *a*, ordinary glass, open bed, yellow, green, blue violet. The development of chlorophyll is favored by a greater and greater removal of the ultra violet. Also chlorophyll decomposition is deferred in old plants by screening out the ultra violet rays.

SCHANZ mentions the fact that in Holland many growers prefer crude glass to regular window glass for forcing houses. He finds that crude glass cuts out more of all rays than window glass, but that it is especially effective in screening out ultra violet, and concludes that any possible detrimental effect from reducing light intensity for synthesis is more than counteracted by the benefits derived from screening out injurious ultra violet rays.

From these results it seems evident that SCHANZ was fully justified in his earlier statement that ultra violet light of the solar spectrum has a remarkable effect on the development of plants.

In his very extensive work KLEBS has always maintained that the action of red light was due to the fact that it has high photosynthetic action. SCHANZ's work suggests that the effect may be due in part to the fact that it eliminates detrimental ultra violet rays.

This very important work of SCHANZ merits checking up and extending. The work with ultra violet light has been largely with artificial spectra much richer in ultra violet than the solar spectrum, and too little exact study has been made of the formative effects of the ultra violet of the latter spectrum.

A very noteworthy piece of work by GARNER and ALLARD,[8] reviewed in detail elsewhere in this journal, should be mentioned in this connection. It is possible that the remarkable effects they obtain from length of day is due to the fact that it modifies the nitrogen *carbohydrate* ratio of which FISCHER, KRAUS and KRAYBILL,[9] and others have made so much as a determiner of the course of development, whether vegetation shall dominate or there shall be a balance of vegetation and reproduction.

In the small dosages of light used in phototropic and photo-growth response, the most effective region of the spectrum on the basis of equal energy value lies at λ 505 $\mu\mu$ for the sporangiophore of *Phycomyces;* at λ 467 $\mu\mu$ for the coleoptile of *Avena;* and at λ 494 $\mu\mu$ for *Volvox,* as previously given. Perhaps with the high dosage of natural illumination the effective region shifts still more to the right as is indicated by SCHANZ's work.

A comprehensive study of the formative effect of light on plants is much needed to see to what degree its formative action is due to synthetic activity, to the so-called photo-growth responses, to various effects of ultra violet rays, and to other effects not included in these.—WM. CROCKER.

Effect of light exposure on plant growth.—GARNER and ALLARD[10] have grown plants under different conditions of light exposure, and have made a special study of the tendency to become reproductive or to remain vegetative under varying daily lengths and intensities of exposure. Several varieties of tobacco and soy bean were mainly used in the experimental work, although numerous other species of annuals and biennials were used to check the results attained.

Plants were grown in pots, buckets, or boxes, and at the desired time each day were moved into dark chambers which were placed in the field. For the last season's work, large dark houses were constructed, in such a way that plants could be moved in or out at any time. Time of exposure to light

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[8] GARNER, W. W., and ALLARD, H.A., Effect of relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agric. Res. **18**:553–606. 1920.

[9] BOT. GAZ. **67**:445–446. 1919.

[10] GARNER, W.W., and ALLARD, H. A., Effect of relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agric. Res. **18**:553–606. 1920.

varied in the different tests from 5 hours daily to full daylight, 7 and 12 hours being the exposures chiefly used. Checks received full daylight under similar conditions of temperature. Shorter light exposures were all made during the middle of the day, and during the time of highest light intensity, except one series of soy beans which were kept in darkness from 10:00 A.M. to 2:00 P.M. daily.

In general, the amount of vegetative growth was proportional to the length of daily exposure to light. The short exposures resulted in short, slender plants of greatly reduced size. Rate of growth was much slower, and the total size attained was reduced. The inception of the flowering or reproductive phase was greatly influenced by length of exposure to light. Many of the species worked with were thrown into flowering and fruiting by the shorter exposures, while with certain other species and varieties, reducing the period of illumination had little effect upon the inception of fruiting.

The authors conclude that for each plant there is a "critical" length of daylight exposure essential to the development of the fruiting phase. The length of this critical exposure varies with each species and variety, but, in many individuals at least, is very much shorter than normal summer daylight. By exposing the plants to this critical length of illumination, the reproductive or flowering phase can be induced at almost any time. By varying this time of exposure, typical biennials, as *Aster linariifolius*, could be made to complete their life cycles within a few months, while annuals, as soy beans, *Solidago*, etc., could be induced to respond as biennials.

Experiments with shading indicated that time of exposure, and not light intensity, is the primary factor involved in determining the critical day. Light intensity reduced to 43 per cent by shading had no effect upon the time of inception of fruiting, although it did give typical shading results on form and amount of growth. Of significance, however, is the result obtained from exposing soy bean morning and afternoon, but keeping it in darkness during midday. Time of fruiting was not materially altered by this treatment, although it was much advanced in the same variety by reducing the exposure to light through leaving in darkness morning and evening. Reducing the water supply reduced vegetative growth and fruit yield, but did not alter time of fruiting in the least. Winter light, supplemented by artificial illumination at night, giving a total daily exposure of 18 hours, acted exactly as long summer daylight in its tendency to retard or prevent fruiting. The authors believe length of day, through its influence on fruiting and seed formation, to be a fundamental factor in plant distribution.

No attempt has been made by the authors to explain how length of day might thus determine the form of plant development. It is unfortunate that a more careful review of the literature was not given, as the authors have made no attempt to link their work to other very critical studies along this line. KLEBS found that by varying the salt nutrients, he could induce vegetative or reproductive growth at will, over a very wide range of plants.

He found high salt supply gives vegetative growth, while low salt supply induces fruiting. FISCHER found that increasing photosynthesis and the supply of carbohydrate material, through increased CO_2 pressure, the nitrogen supply remaining constant, induces the reproductive phase. Finally, in a series of very critical experiments and analyses, KRAUS and KRAYBILL found that a relative abundance of carbohydrate over nitrogenous material in the plant induces fruitfulness, while a relatively greater nitrogen supply induces vegetative growth. Excessive carbohydrate over nitrogen inhibited both vegetative and reproductive growth. All of this work shows a very close relation between the conditions of nutrition in the plant and the type of growth expression. GARNER and ALLARD have undoubtedly made a contribution of great value to the subject of vegetation and reproduction in plants. The reviewer feels, however, that their conclusions are much broader than a careful review of the whole subject warrants. It would be difficult, for example, to explain the phenomenon of alternate fruiting in many of our orchards on the basis of length of day influence. A critical study of the nitrogen and carbohydrate metabolism under these reduced exposures to illumination would be of great value in arriving at an understanding of the many factors in plant growth and reproduction.—J. R. MAGNESS.

Ecological research.—In a report of research in progress under the direction of the Carnegie Institution, Director MACDOUGAL[11] reports progress upon a number of interesting problems. SHREVE has continued investigations upon the vegetation of the arid Avra Valley, and reports progress in a soil temperature survey of the United States and Canada. CANNON presents some conclusions derived from a field study of the vegetation of central, northern, and southwestern South Australia, as well as some further results in the investigations of the reactions of roots to varying amounts of carbon dioxide in the soil. He has also some data as to the size and form of leaves of desert plants. COOPER reports the beginnings of a study of the strand vegetation of the Pacific Coast at Coronado and Monterey, California. These regions possess interesting dune areas, upon which various plant associations, varying from pioneer herbaceous to chaparral and forest communities, have become established. These communities are being mapped, permanent quadrats and transects established, and the underground portions of many species excavated and studied. Measurements of evaporation, soil moisture, and soil temperature have been made, and material collected for anatomical studies. MACDOUGAL and SPOEHR are conducting investigations to discover the origin of xerophytism in plants, and Mrs. SHREVE has records extending over several years of seasonal changes in the water relations of such desert plants as *Encelia farinosa*, *Streptanthus arizonicus*, and *Amaranthus Palmeri*.—GEO. D. FULLER.

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[11] MACDOUGAL, D. T., Ecology. Carnegie Inst. Wash. Year Book for 1919. 18:87–102. 1920.

VOLUME LXX NUMBER 4

THE

BOTANICAL GAZETTE

OCTOBER 1920

PHYSIOLOGICAL ISOLATION BY LOW TEMPERATURE IN BRYOPHYLLUM[1]

C. M. CHILD AND A. W. BELLAMY

(WITH SIX FIGURES)

Introduction

It has long been known that certain relations of dominance or control and subordination exist between different parts of plants. Among these relations the most general is the dominance of the chief growing tip over other buds, branches, etc.; that is, over other growing tips. That these relations are not specific for particular parts of the plant is shown by the fact that the growth and development of a bud, for example, may be inhibited by the chief growing tip of the plant, by a branch, or by a leaf. Moreover, the fact has been established that the dominance of one part over another is associated in some way with the metabolic activity of the dominant part. When this is inhibited, for example, by inclosure of a growing tip in an atmosphere without oxygen, or in plaster, the effect on other buds is essentially the same as if the growing tip had been removed; that is, it is no longer dominant, but may regain its dominance when the inhibiting conditions are removed and it again becomes active.

In various publications (CHILD 1–6) it has been pointed out that this relation of dominance and subordination is not peculiar to plants, but that similar relations exist in animals. In these

[1] From the Hull Zoölogical Laboratory, University of Chicago.

and other papers it has also been maintained on the basis of various lines of evidence that this relation originates in a difference in rate of fundamental metabolic reactions, such differences of course being associated with differences in protoplasmic condition. These differences appear in the form of gradations in physiological condition, which have been called metabolic or physiological gradients. Since a discussion of the nature and origin of these gradients and the evidence on which the conclusions are based has recently appeared (CHILD 6), extended discussion of these matters is not necessary at this time. In such a physiological gradient the dominant region is primarily the region of highest metabolic rate, of greatest physiological activity. The evidence indicates further that the relation between dominant and subordinate parts is primarily transmissive, rather than transportative in character; that is, that the dominance of a particular part is primarily dependent upon dynamic changes transmitted from it to other parts, rather than upon the transportation from the one part to the other of substances in mass. Since this dynamic effect of a region of high metabolic rate upon other parts is a factor in determining the metabolic rate of the parts affected, and since in protoplasm without highly specialized conducting paths it decreases with increasing distance from the region of high rate, the result of the establishment of such a region of high rate, however brought about, is the development of a more or less definite physiological gradient. The physiological axis in its simplest terms is such a gradient, determined in relation to a region of high activity which is the dominant region of the axis.

It has been found, largely through the work of HYMAN and BELLAMY, which is not yet published, that gradients in electrical potential and electric currents resulting from them are characteristic features of these physiological gradients. Discussion of the significance of these bioelectric currents is impossible here, but many facts make it highly probable that they are the factors primarily concerned in transmission of excitation (LILLIE 8–13), and that they play a fundamental part in determining and maintaining the physiological gradients which arise in relation to regions of high metabolic rate. If this is true, the dominance of

one part over another is probably primarily a matter of the differences in electric potential and resulting currents. In general, the "high end," the dominant region of a physiological gradient, is externally electro-negative to other levels of the gradient, and in this respect it is similar to a region of excitation, which is also externally electro-negative to less excited or unexcited regions. From this viewpoint the physiological gradient may be regarded, at least tentatively, as the physiological expression or effect of the potential gradient and the resulting currents which arise in relation to a region of high metabolic rate. In fact, the physiological gradient in its simple form shows all the characteristics of an excitation-transmission gradient in protoplasm. In all except the simplest animals a nervous system with definite morphological conducting paths develops as an expression and resultant of the physiological gradients, and after definite nervous relations are established between parts, the dominance of a particular region, for example, the head, is no longer necessarily dependent upon the persistence of the metabolic conditions which originally determine its dominance. While the rate of metabolism concerned in the initiation of a nervous impulse is undoubtedly high, its total amount may be exceedingly small, yet the impulse may determine an enormous amount of metabolism in the organ affected by it.

In plants, however, no nervous system develops, and the relations of dominance and subordination apparently depend throughout life upon essentially quantitative physiological differences of the same sort as those in which the relation originates. The nervous structure of higher animals is capable of conducting impulses for long, perhaps for indefinite distances; but in the less highly specialized protoplasms of the simpler animals and the plants the dynamic effects of excitation undergo a decrement with increasing distance from their point of origin. Such a decrement determines the existence of the physiological gradient, and it is evident that under such conditions physiological dominance of any part must be limited in range, and that therefore the possibility of what the senior author has called physiological isolation (1–6), that is, of escape or isolation from such dominance without physical separation of parts, exists. Theoretically physiological isolation

may occur in four ways: first, growth in size of the organism may bring some part of it beyond the range of the dominant region; second, since dominance is primarily dependent upon the metabolic activity of the dominant region, a decrease in this activity, however brought about, will decrease the range of dominance and may bring about physiological isolation in more distant parts without increase in size; third, if physiological dominance is dependent upon transmission of electric or other dynamic effects through protoplasm, physiological isolation must result from blocking the passage of such effects; fourth, the subordinate part may be directly excited by external factors to such a degree that the action of the dominant part upon it is no longer effective, for example, stated in electrical terms, it may itself give rise to electric currents in the opposite direction from those in the dominant region and compensating them.

The physiologically isolated part behaves essentially as it would if the dominant part had been removed, or it itself separated from the dominant region. If its growth and development have previously been inhibited, it begins to grow and develop. If it represented a differentiated part of the body, as in many animals, it reacts in the simpler forms by losing this differentiation and may give rise agamically to a new individual.

Physiological isolation and consequent development of new parts or individuals as a result of growth is a familiar phenomenon in both plants and animals. The experimental decrease in the activity of the growing tip by inclosing it in an atmosphere lacking oxygen, or in plaster, as well as many cases of the inhibiting action of external factors in nature on growing tips afford numerous examples among plants of the second kind of physiological isolation. The fourth type of isolation appears in cases in which a bud may be made to grow in spite of the inhibiting action of a growing tip or other part, by subjecting it to external conditions which increase its activity. Such isolation may be brought about in some plants, particularly in the buds farthest away from the dominant region. In *Bryophyllum*, for example, the buds in the notches of the lower leaves will often develop under favorable external conditions.

As regards the third type of physiological isolation, by the blocking of passage of the action or effect, whatever its nature, less is known. McCallum (**21**) obtained some results along this line in plants by means of local anaesthesia, and it is a familiar fact that the passage of the nerve impulse can be blocked by an anaesthetized or a cooled region, as well as by various other means. For a long time one of us has had in mind experimentation along this line with plants, and Loeb's (**14–20**) recent work on *Bryophyllum* constituted an additional stimulus. Finally, in 1918–1919, experimentation was begun in the attempt to determine whether physiological isolation could be brought about by a local reversible action on some part of the connecting path between dominant and subordinate parts. Since it seemed desirable to avoid the use of anaesthetics or other chemical agents which might enter the plant tissues and be transported in one direction or another, low temperature was used as the blocking factor.

Method and apparatus

The low temperature was obtained by a current of cold water flowing through a block tin pipe of $\frac{1}{8}$ or $\frac{3}{16}$ inch inside diameter, this pipe being bent into loops or coils of the proper diameter and length to surround the portion of the plant to be cooled. In this way various lengths of stem, from 1 cm. to a whole internode or more, could be cooled. In preparation of the plant, the region to be subjected to the low temperature was first wrapped in tinfoil, and the loop or coil of pipe, supported by clamps attached to ring stands, was fitted about it in such manner that it was not in direct contact with the plant at any point, the space between stem and pipe being usually 0.5–1 cm. This space was then packed lightly with moistened absorbent cotton to keep the temperature as constant as possible, and finally the whole coil was wrapped closely in non-absorbent cotton to protect it from the outside temperature. In many experiments a thermometer was also inserted in the coil, but it was found that so long as the temperature of the water passing through the coil remained constant there was practically no change inside. In a part of the experiments the temperature of the water used was maintained in a tank by

means of a refrigerating brine coil under thermostatic control. The freezing point of the water in the tank was slightly lowered by the addition of alcohol in order to avoid accumulation of ice about the brine coil. The circulation of water from and to the tank was maintained by an electrically driven pump. The flow from the tank was led to a horizontal feeder pipe 6 ft. above the experimental table, and in this feeder six outlets were tapped about 30 cm. apart. At the table level a similarly tapped collector pipe returned the water to the tank. The flow for each plant was led from the feeder by one of the six insulated outlets, through rubber tubing to the loop or coil about the plant, through the coil and back to a corresponding tap on the collector pipe. With this apparatus as many plants as there were pairs of outlets (supply and return) could be placed under experimental conditions at the same time. Each supply pipe was fitted with a valve, making it possible independently to regulate or stop entirely the flow of cold water through any of the six coils. By means of these valves temperatures differing by several degrees could be maintained in different lines without affecting appreciably the temperature of the general supply. All exposed metal piping was covered with non-conducting material. During the winter months, while the temperature of the city water was so low that it could be used directly, a second similar system was also arranged for use with the city water. Each system was supplied with six connecting lines, so that twelve experiments could be conducted at the same time. With a little care it was possible to control within about 1° C. the temperature to which the cooled region was subjected. This temperature apparatus was devised by the junior author, and for its maintenance in proper working order during the experiments he is largely responsible.

The temperatures found to be effective for the purpose ranged from 2.5 to 6° C., according to the species of plant used and the region subjected to cooling. In the bean seedling, in which regions of the main stem were cooled, the more basal levels of the stem required a lower temperature than more apical levels to bring about physiological isolation and outgrowth of buds below the cooled zone.

Thus far experiments have been made chiefly with three species of plants: *Bryophyllum calycinum*, in which physiological isolation and outgrowth of buds in the notches of the leaves was brought about by cooling a region of the petiole; seedlings of *Phaseolus multiflorus*, the scarlet runner bean, in which isolation and outgrowth of axillary buds were brought about by cooling a region of the main stem between the buds to be isolated and the chief tip of the plant; *Saxifraga sarmentosa*, in which the isolation and development of the runner tip into a new plant was brought about by cooling a zone of the runner below its tip. A brief report of the results of these experiments has already appeared (CHILD and BELLAMY 7). The present paper is devoted to the experiments on *Bryophyllum*.

Experiments

The individual plants used ranged from 0.6 to 1.3 m. in height. Leaves from the upper half of the plant were selected for experiment in nearly all cases, particularly in the larger, older plants, in which the lower leaves are often in poor condition, or, when the plants have been kept in moist air, show more or less outgrowth of the buds during the winter in the intact plants. Various preliminary experiments were performed in order to determine to what extent physiological isolation might be brought about by external conditions acting directly on the leaves while still attached to the intact plant. By arranging bowls or jars containing water on ring stands about the plant in such a way that particular leaves were more or less completely submerged, it was found that at least during the winter and early spring months some of the leaf buds would grow in some cases, but to a greater extent on leaves at lower than on those at higher levels of the plant.

Again, a sudden rise in temperature from 15 to 25° C. in saturated atmosphere would usually induce outgrowth of some buds on leaves of the middle and lower levels of the plant, but not on those near the main tip. Such outgrowth usually consisted only of roots, and these were often inhibited after a few days.

Direct injury to the petiole, for example, compression by a screw clamp or by a cut partially through the petiole, was usually

effective, if sufficient in degree, in inducing more or less outgrowth of leaf buds, provided the leaf concerned was in saturated air or water. Slight superficial injury, even though it extended completely around the petiole, had little or no effect. An injury to the petiole of one leaf sufficient to induce outgrowth of some of the buds of that leaf usually induced outgrowth of some buds on the opposite leaf, and sometimes also on leaves of the next node above or below, if these were in moist air or water. Such mechanical injury by partial section of the petiole or by compression, however, was less effective than low temperature, unless the injury was sufficient to interrupt physiological continuity to a very considerable degree. Cuts half way through the petiole, for example, brought about development of some buds on the leaf, but usually of only a few, and in the case of lateral cuts the buds growing were not necessarily on the same side as the cut. Mechanical compression of the petiole by a screw clamp gave similar results. Fig. 1 shows a case in which the petiole of the leaf *A* was compressed to half its thickness by the screw clamp. The leaf *A*, the opposite leaf *B*, and one leaf *C* of the next pair below were partially submerged. On leaf *A* five buds grew out of eighteen submerged; on leaf *B*, seven out of seventeen submerged, but they are less advanced than those of *A*. On leaf *C* there is slight growth of four out of seventeen buds submerged, but this growth consists merely of short roots and is not visible in the figure. In another similar experiment slightly less compression resulted in outgrowth of only three buds out of twelve submerged on the leaf with compressed petiole, and no growth in *B* and *C*. Comparison of these results with those obtained by low temperature described later

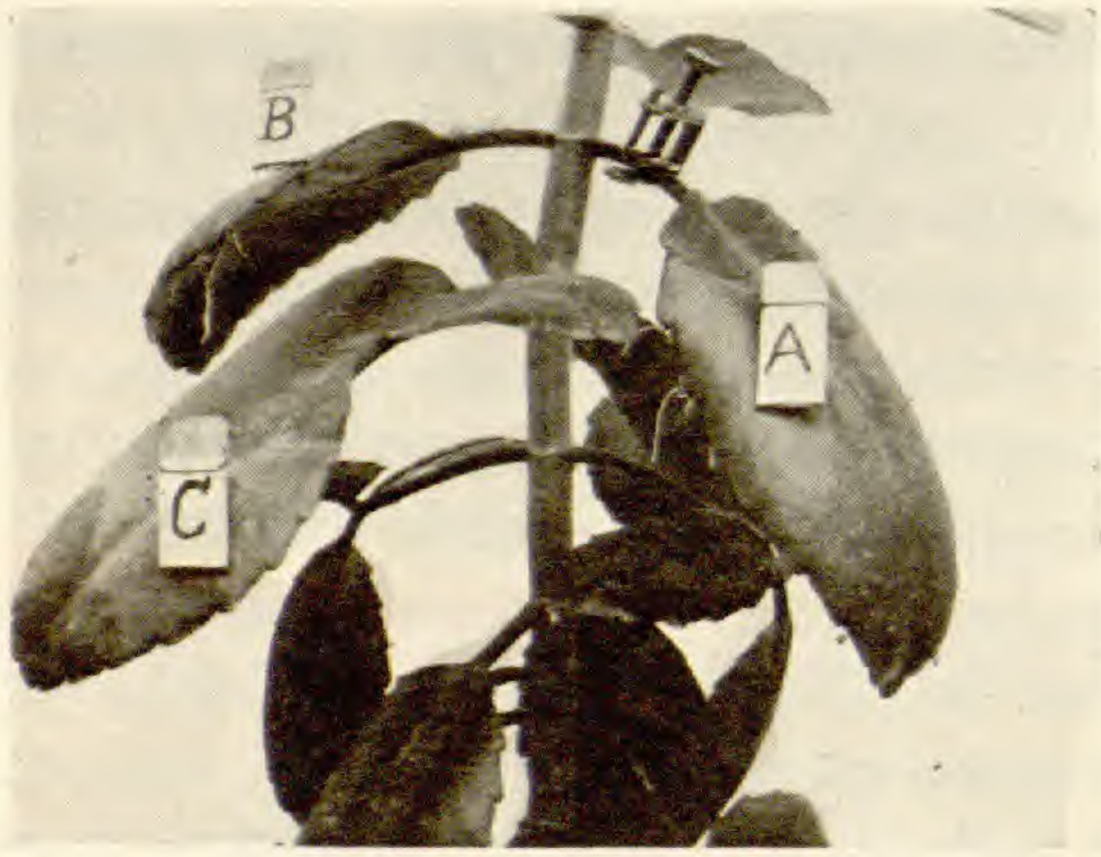

FIG. 1

shows that the low temperature is far more effective, even though no visible injury results, than mechanical compression.

These various experiments show very clearly that the dominance of the chief growing tip of *Bryophyllum* may be overcome to some extent at levels below the most apical five or six nodes without separating the leaf from the plant or inhibiting the chief tip, and in some cases by merely placing the leaf in water or in moist air with its petiole and attachment intact. Such isolation, however, usually results in development of only a part or a few, often of only one or two of the buds on a leaf. Whether physiological isolation of the leaf buds will occur as readily during the summer months has not yet been determined. The fact that injury to the petiole of one leaf may, if sufficient in degree, induce growth of buds in the opposite leaf and often in the leaves of adjoining nodes, shows further that the inhibition of growth of buds in any leaf is due, not merely to the chief tip of the plant, but to the opposite leaf and to some extent to other leaves also. This fact has also been shown by earlier work and more recently by LOEB's experiments.

The results of cooling a zone of the petiole, however, are much more striking. In these experiments a portion of the petiole 2–3 cm. in length is subjected to the low temperature, the rest of the petiole and leaf being exposed to room temperature, and the leaf blade more or less completely submerged in water, as indicated in fig. 2, or by placing an open bowl of water in such position that the leaf rests in the water. The succulent tissues of *Bryophyllum* are very susceptible to injury by continued pressure, and care must be taken that the low temperature coil does not touch the petiole, that the packing of the coil is not too tight, and that the petiole does not touch the edge of the jar or bowl in which the leaf is submerged. In some experiments, particularly the earlier, injury of the petiole resulted from one of these causes; but although the results of the experiments on mechanical injury indicate that the slight injuries thus produced had little or no effect on the leaf buds, only those experiments in which no visible mechanical injury of the petiole was found after removal of the low temperature zone are regarded as entirely satisfactory.

In room temperature of 20–25° C., growth of the leaf buds usually became visible three to four days after the low temperature coil was placed, the first indication being the outgrowth of one or

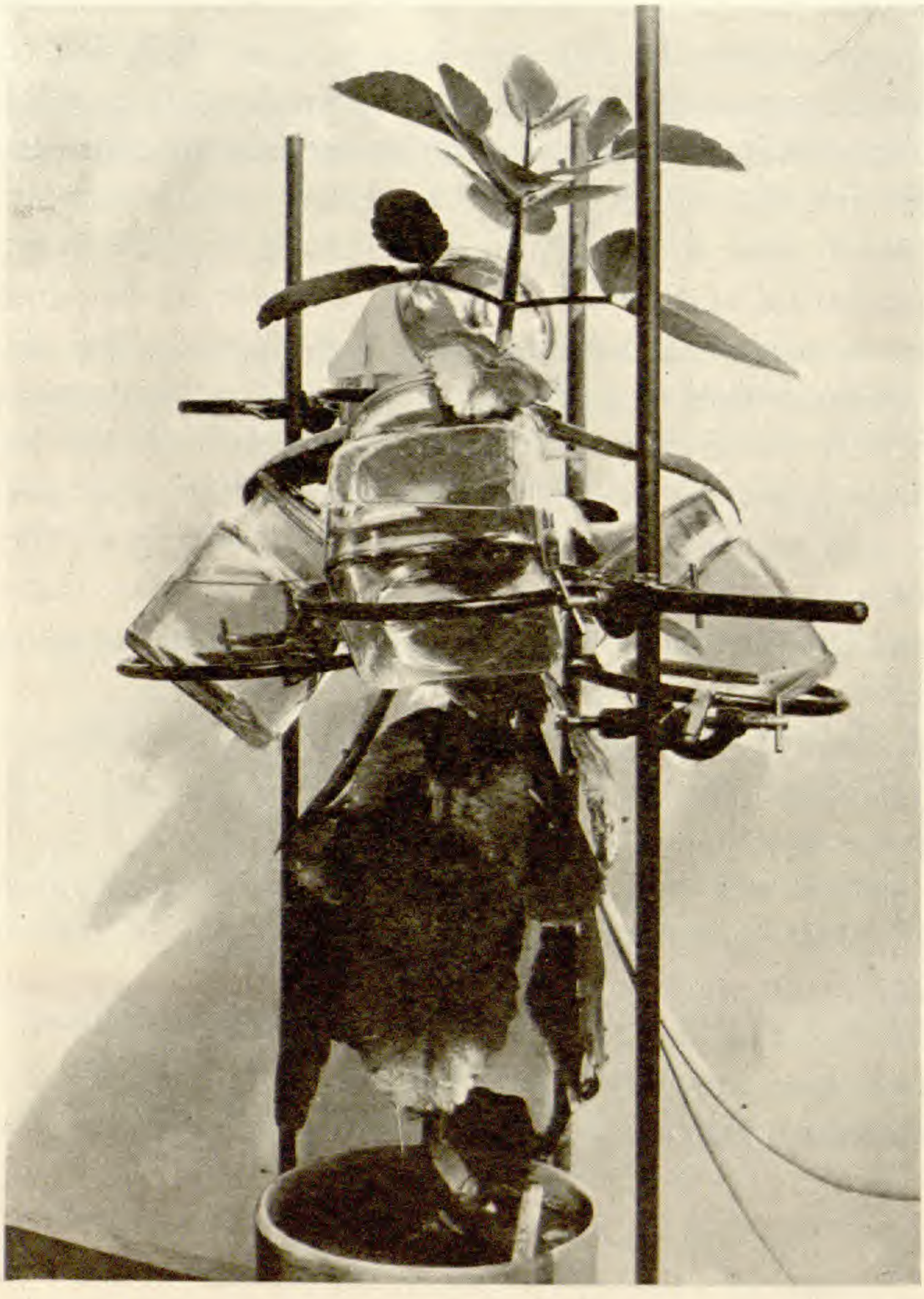

FIG. 2

more roots. If the low temperature coil was removed at this time, shoots usually did not appear, or appeared in only a few notches, the others being inhibited. If the low temperature coil remained in place six to eight days, shoots as well as roots were

usually clearly visible before its removal, and growth always continued afterward in at least a considerable number of notches. When the coil remained on the petiole for ten days or more, death of some of the epidermal cells usually occurred, although internally the petiole might be completely intact and to all appearances in good condition. Probably such superficial injuries were without effect, but cases in which they occurred were not regarded as conclusive.

In all, twenty-eight low temperature experiments were performed with *Bryophyllum* leaves attached to the plant. In fifteen of these there was some injury to the petiole, but in the other thirteen no injury was apparent. In all these experiments not only the experimental leaf, but the opposite leaf, and usually one or more leaves of nodes above and below the node of the experimental leaf, were more or less completely submerged, in order to determine to what extent these leaves were also affected. Usually all, or nearly all, buds on the submerged portion of the experimental leaf and in most cases those of the leaf opposite developed. On some leaves individual buds had been killed or injured by plant lice, from which the plant could not be kept entirely free. As far as possible, leaves were selected for experiment on which all the buds were apparently intact, but occasionally such buds failed to grow.

In order to determine whether cooling of a zone of the petiole stopped the flow of water to the leaf, experiments were performed in which the experimental leaf remained in air of medium humidity, instead of being submerged. Such leaves did not wilt, but remained fresh and in good condition, while leaves separated from the plant and exposed to the same atmosphere showed distinct wilting in the course of a few days. Evidently the cooled zone does not appreciably affect the flow of fluids to the leaf. The following descriptions and figures of typical experiments will serve to show the results attained.

Series 35, March 12, 1919.—Temperature of 2.5–3° C. was placed on petiole of one leaf of the eighth pair below the tip. The terminal leaflet of this leaf, of the opposite leaf, and one leaf of the ninth pair were submerged. Growth was visible in both leaves

of the eighth pair after three days, but none in the leaf of the ninth pair. After five days the cooled zone was gradually brought to room temperature and the coil removed. Fig. 3 shows the condition of the plant twelve days after the beginning of the experiment. The experimental leaf is the member on the left of the figure of the pair showing bud development. In this and the opposite leaf all buds which were submerged show vigorous outgrowth. In the leaf of the ninth pair ten buds out of twelve submerged developed, but only three of these produced distinct shoots, and all are much retarded as compared with those of the leaves above. Fig. 3 does not show the roots on this leaf, since they are on the under side.

FIG. 3

Series 44, March 30, 1919.—Temperature of 3–4° C. on petiole of one leaf (*A*) of seventh pair below tip. Opposite leaf (*B*) and both leaves (*C* and *D*) of sixth pair in water. The leaf *D* was in water eight days preceding the temperature experiment, but during that time showed no development of buds. After seven days the cooled zone was gradually brought to room temperature and the coil removed. The experimental leaf (*A*) showed growth in ten notches, *B* in two notches, *C* in none, and *D* in five notches. During the following week three days of high temperature in the greenhouse occurred, and this may have aided the development of some further buds. The condition of the four leaves eighteen days after the beginning of the experiment is indicated in fig. 4. In *A* every bud submerged has developed; in *B* nine buds out of fourteen submerged have developed, but only three of them have gone beyond the earliest stages; in *C* eight buds out of sixteen submerged show some development, but all except two have been inhibited in early stages; in *D* four buds out of fifteen submerged have developed

and show shoots as well as roots. Fig. 4 shows clearly the difference between *A* and the other leaves, although it does not show all the development in *B*, *C*, and *D*. The more advanced development of the four buds in *D* suggests that the eight days in water preceding the temperature experiment may have had some slight effect in the way of isolation, although it did not lead to visible development.

Series 45, April 4, 1919.—Temperature 2 . 5–3° C. on one leaf (*A*) of sixth pair below tip; *B*, opposite leaf, *C*, one leaf of seventh pair, *D*, one leaf of eighth pair, and *E*, one leaf of fifth pair also in water. After seven days the cooled zone was gradually brought to room temperature and the coil removed. Fig. 5 shows the plant seventeen days after beginning of the experiment. In *A* fourteen buds out of fifteen submerged developed and formed vigorous shoots; in *B* seven out of fifteen submerged developed to some extent, but produced only roots; in *C* eleven out of fifteen submerged showed some development, but only two produced shoots; *D* showed no growth; in *E* all buds submerged (thirteen) developed, but only six produced shoots. The growth on leaf *C* is not well shown in the figure. The slight development in the leaf *B* of this experiment is unusual. In most cases the mate of the experimental leaf shows almost or quite as much growth as the experimental leaf itself.

FIG. 4

Series 43, March 27, 1909.—Temperature of 2 . 5–3 C. on petiole of one leaf (*A*) of eighth pair; only the terminal leaflet in water; *B*, leaf opposite *A*, *C*, one leaf of eleventh pair, *D*, *E*, terminal leaflets of leaves of ninth pair, *F*, one leaf of seventh pair, *G*, one leaf of fifth pair, all in water. After six days the cooled zone was gradually brought to room temperature and the coil removed.

At this time *A* showed the greatest development, both as to stage and number of buds (eight) developing; *B* showed two buds developing; *C* and *D* none; *E* six; *F* one; and *G* none. Fig. 6 shows the plant fifteen days after beginning of the experiment. At this time in *A* seventeen buds out of nineteen had developed, and sixteen had produced shoots as well as roots; in *B* fourteen buds out of sixteen submerged had developed, thirteen with both shoots and roots; in *C* four buds out of nineteen submerged had developed, all with both shoots and roots; in *D* two buds out of ten

FIG. 5

FIG. 6

submerged had developed, one with shoot and roots; in *E* ten buds out of sixteen submerged had developed, all with shoots and roots; in *F* eight buds out of sixteen submerged had developed, three shoots and roots, five roots only; in *G* seven buds out of twelve submerged showed some slight development, but only two showed shoots, the others roots only.

In this and other experiments described, some of the isolations on leaves distant from the experimental leaf may not be the result of the cooling, but merely such as occur on most plants at this season, when leaves are submerged. In all the series, however, it is evident that the greatest degree of isolation, both as regards

number of buds developing and degree of development, has occurred in the experimental leaf and the leaf opposite.

In these experiments generally the buds which do not develop distinct shoots as well as roots within a week, either do not develop shoots at all, or give rise to shoots which grow very slowly and often soon cease to grow. Such buds are evidently inhibited to a greater or less degree. Apparently they represent somewhat less active or weaker buds, which react less rapidly than others to the isolating conditions, and so do not advance far enough before the return of the experimental leaf to normal conditions to maintain their growth afterward.

It appears true also that in general buds give rise only to roots when the degree of physiological isolation is relatively slight, while with more complete isolation shoots as well as roots appear. The length of the isolation period is probably also a factor, since the outgrowth of roots begins somewhat earlier than that of the shoots, or at least occurs more rapidly during the early stages. A brief period of isolation gives time for roots to appear, but a longer period is apparently necessary for the shoot to become well started.

Discussion

It is evident from these experiments that a mere cooling of a zone of the petiole of the *Bryophyllum* leaf without visible physical injury serves to block the inhibiting action of the chief growing tip and other parts upon the buds of that leaf, and also the inhibiting action of the leaf upon the buds of the opposite leaf and other leaves in the same region of the plant. That this cooling does not block the flow of fluids and substances in solution through the vascular bundles is indicated by the experimental fact noted that leaves show no wilting in an atmosphere in which leaves severed from the plant wilt. In the case of the bean seedling, to be discussed in a later paper, this is still more clearly evident, for there the zone of low temperature is placed about the main stem, and all substances passing from below to those parts of the plant above the cooled zone must of course pass through it. In such cases there is no wilting of the parts above the zone, and growth is either not at all inhibited, or, when the region cooled is young

and not fully developed, growth of the parts above may be retarded slightly for two or three days, but soon proceeds normally. These facts do not support the view which LOEB has advanced that the inhibiting action of the growing tip and of other parts on buds is due to the transportation of inhibiting substances through the regular channels of transportation in the plant. In these experiments such transportation is not appreciably or only very slightly affected, yet the zone of low temperature is much more effective as a means of physiological isolation than mechanical compression or partial section of the petiole, except when these involve the greater part of the petiole tissues. Unless we assume that the hypothetical inhibiting substance in some way is rendered inactive by the short cooled zone, we must conclude that the dominance of the growing tip and of other regions over a particular leaf is not dependent upon the flow of substances through the vascular bundles to the leaf, but rather upon some sort of action which is dependent upon the physiological activity of the cells. When this activity is inhibited by the low temperature, the action is blocked, unless and until some degree of acclimation of the cooled zone occurs. Such acclimation occurs very readily in the bean seedling, and in many cases a temperature which at first serves as a block becomes ineffective after a few days. In short, the experiments indicate that the physiological dominance of one region over another in these plants is dependent on some sort of effect transmitted physiologically through the living active protoplasm, rather than upon substances transported by the flow of fluids.

LOEB appears not to distinguish clearly two different aspects of the relations of parts: the one which is concerned with the conditions that prevent or permit the initiation of development and growth in a subordinate part; the other which is concerned with the amount of growth or development of the part which may occur after its initiation. Nutritive factors may play a large part in determining the amount of growth of buds, but there are no reasons for and many against maintaining, as LOEB did in earlier papers, that they initiate it. Again, the mass of shoots and roots developing from an isolated *Bryophyllum* leaf may show a certain proportion to the size of the leaf, since the amount of certain

nutritive substances available must depend upon the size of the leaf, but such relation tells us nothing concerning the factors which initiate the development.

On the other hand, the assumption of the transportation of inhibiting substances, made in LOEB's later papers, also involves certain difficulties. In the first place, each part which produces such substance or substances must be immune to the action of the substance which it produces, since it is not inhibited by it, yet in the case of growing tips the substance produced by one growing tip inhibits other tips. This presupposes a remarkable specificity of action on the one hand and absence of specificity on the other, and it is difficult to conceive how the hypothetical substance could possess the properties required. Certain assumptions concerning the direction of flow of the inhibiting substances also have no basis in fact and do not agree well with the facts at hand. Certain other objections to the assumption of inhibiting substances scarcely require discussion in view of the work of various botanists and the experiments just described.

In an early paper LOEB (**14**, pp. 251–253) endeavored to show that isolation is not the initiating factor in the outgrowth of buds on the leaf of *Bryophyllum*, and described three experiments to prove his point (see his *figs. 1, 2, 3*). In the first a leaf partially submerged is completely separated from the stem; in the second it remains attached to a piece of stem cut off above and below the node and the opposite leaf is removed, but its axillary bud remains; and in the third the opposite leaf also remains. In the first experiment and in the third the submerged buds develop, in the second they do not, but the axillary bud of the opposite side develops in the absence of its leaf. LOEB maintains that the leaf in the second experiment is more isolated than in the third, but its buds do not grow out, therefore isolation cannot be the factor determining the development of the buds. This conclusion is incorrect and based upon a misconception of isolation. Actually the leaf of the second experiment is less isolated than in the first and third, because in this experiment the axillary bud of the opposite side develops and inhibits the leaf buds. If this growing tip is removed, the buds of the leaf will develop. In the third experiment the

axillary bud is inhibited by its own leaf and there are no active growing tips to inhibit the direct action of the water in inducing bud development in the experimental leaf. Isolation from an active growing tip is the chief factor in the development of the leaf buds, and such isolation exists to a greater degree in LOEB's first and third experiments than in the second, in which the axillary bud of the opposite side starts before the leaf buds of the experimental side. These three experiments, therefore, instead of disproving, as LOEB asserts, that isolation is the factor initiating development of the leaf buds, constitute evidence in support of the conclusion that it is such a factor. Moreover, according to LOEB's later assumption of inhibiting substances, it would seem that isolation must be the initiating factor.

The chief results of the paper are summarized as follows. The cooling of a zone of the petiole of the *Bryophyllum* leaf to a temperature of 2.5 to 4° C. for a few days is a very effective means of inducing the outgrowth of the leaf buds. Usually the opposite leaf and often leaves of adjoining nodes also show more or less development. The passage of fluids to the leaf is not appreciably interfered with by the cooled zone; therefore it seems improbable that physiological isolation of the leaf can be due to the blocking of passage of inhibiting substances transported in these fluids.

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SWELLING OF AGAR IN SOLUTIONS OF AMINO ACIDS AND RELATED COMPOUNDS

D. T. MacDougal and H. A. Spoehr

(with six figures)

A study of the behavior of a large variety of substances in aqueous solution on the hydration of agar has shown that there are very few such solutions in which agar swells to a greater degree than it does in distilled water. There are some substances, however, which increase the hydration of agar above that attained in pure water. These are the amino acids. The amino compounds are of such immediate biological importance that a discussion of their action deserves special consideration, and may aid in explaining the scattered results obtained by various workers in which increased total growth and apparently catalytically accelerated actions have been obtained by the addition of certain amino acids to culture solutions.

The purification of the agar, as well as the preparation of the agar plates and the instruments and methods for measuring the swelling, have already been described in detail by MacDougal.[1] The results here discussed were obtained by the application of these methods. The amount of swelling in water is taken as the standard. Thus the percentage of swelling of the dried plates in water is expressed as 100, and that in the various solutions is calculated on this basis.

Swelling of agar in solutions of amino acids

The dried agar plate, prepared from the specially purified agar and used in this experiment, showed a total swelling in distilled water of 2000 per cent. The amino acids, glycocoll, alanin, and phenylalanin were used in 0.01 normal concentration. The solutions in which the agar plates were allowed to swell were renewed every 24 hours; the swellings were complete after about 6 days. The results are given in table I.

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In table II are given the results of some earlier experiments made with plates prepared from the "bacto-agar" of the Digestive Ferments Company. The swellings in the solutions of the corre-

TABLE I

SWELLING OF AGAR PLATES 0.11 MM. IN THICKNESS AT 15°C. IN 0.01 NORMAL SOLUTIONS OF AMINO ACIDS, CALCULATED ON BASIS OF SWELLING IN WATER TAKEN AS 100; SOLUTIONS RENEWED EVERY 24 HOURS; SWELLING OF AGAR PLATES IN WATER 2000 PER CENT

Water	Glycocoll	Alanin	Phenylalanin
100.....	165	151	161

sponding organic acids are also given for comparison. Thus it can be seen that in acetic acid agar exhibits a much lower swelling than in the a-amino compound, glycocoll. The same relation is maintained with the other acids.

TABLE II

SWELLING OF AGAR PLATES 0.10–0.23 MM. IN THICKNESS AT 16–17°C. IN SOLUTIONS OF AMINO ACIDS AND CORRESPONDING ORGANIC ACIDS, CALCULATED ON BASIS OF SWELLING IN WATER TAKEN AS 100; DRIED PLATES SWELLED 2570 PER CENT IN WATER; DURATION OF SWELLING 20–60 HOURS, DURING WHICH TIME SOLUTIONS WERE NOT RENEWED

Normal concentration	Water	Glycocoll	Acetic acid	Alanin	Propionic acid	Asparagine	Aspartic acid	Succinic acid
0.01.....	100	115	61	94	51	91	49	49
0.002.....	100	123	59	108	63	94	58	62
0.0004.....	100	103	76	.....	70	106	69	68
0.00008.....	100	.....	102	.....	.....	126	81	98

It is worthy of note that agar behaves quite differently from gelatine in relation to acids and bases, and that this also applies to the amino acids, as shown in table III. Agar is a carbohydrate, and as such exhibits some of the properties of an exceedingly weak acid. DR. MCGEE of this laboratory determined by means of the indicator method[2] that in 0.75 per cent solution the purified agar shows a hydrogen ion concentration expressed by $P_H = 6.5$.

[2] DUGGAR, B. M., The use of the colorimeter in the indicator method of H ion determination with biological fluids. Ann. Mo. Bot. Gard. **6**:61–70. 1918.

———, The micro-colorimeter in the indicator method of hydrogen ion determination. *Ibid.*, **6**:179–181. 1919.

It has long been known that carbohydrates form salts with metals, and that they react with the chlorides, sulphates, and other salts of the heavy metals, such as copper, lead, mercury, gold, iron, silver, etc., to form the corresponding carbohydrate salt with

TABLE III

SWELLING OF DRIED GELATINE PLATES 0.5 MM. IN THICKNESS AT 16–17°C.; SWELLING IN WATER 600 PER CENT

Normal concentration	Water	Propionic acid	Alanin
0.1	100	.....	91
0.05	100	256	83
0.01	100	185	88
0.002	100	130	86
0.004	100	83	75

the liberation of hydrochloric, sulphuric, or other acids. McGEE has determined the hydrogen ion concentration of a number of heavy metal salts in 2 per cent aqueous solution, and also in mixtures of 2 per cent of the salts plus 2 per cent d-glucose. A 2 per cent solution of d-glucose in water showed $P_H = 6.6$.

TABLE IV

HYDROGEN ION CONCENTRATION EXPRESSED AS P_H OF 2 PER CENT SOLUTIONS OF SOME HEAVY METAL SALTS AND OF SAME MIXED WITH 2 PER CENT D-GLUCOSE

$ZnCl_2$	6.3	$AgNO_3$	5.5	$HgCl_2$	3.9	$CuSO_4$	4.6
$ZnCl_2$ + glucose ...	5.4	$AgNO_3$ + glucose...	5.3	$HgCl_2$ + glucose...	3.8	$CuSO_4$ + glucose...	4.4

It is apparent that in the addition of the glucose to the heavy metal salts the acidity of the mixture is appreciably raised.

The amino acids being amphoteric electrolytes, it is to be expected that they would behave like acids toward bases, and like bases toward acids. Furthermore, there are a number of reactions of which the amino acids are capable which may be of importance in interpreting their behavior toward agar. Thus the simplest amino acid, glycocoll (NH_2CH_2COOH), can apparently give rise to an internal salt, NH_3CH_2COO. Glycocoll in solution would then exist in equilibrium as the un-ionized

NH_2CH_2COOH together with its ions and with NH_3CH_2COO, as well as a hydrated form $OHNH_3CH_2COOH$. The existence of this latter compound has been used to explain why acids such as glycocoll do not follow the simple Ostwald dilution law. The recent observations of BIRCKNER[3] on the interaction of ethyl alcohol and certain amino acids may be interpreted in favor of the theory of salt formation of the alcohol with the amino group. Further insight as to whether the increased swelling of agar in amino acids is due to simple salt formation or to the formation of a compound related to the form $OHNH_3CH_2COOH$ was sought in a study of the behavior of agar toward ammonium hydroxide and some related substances.

Swelling of agar in alkaline hydroxides and in ammonium salts

A study of the behavior of agar in solutions of various alkaline hydroxides revealed a number of facts worthy of notice. The first experiments were conducted in the usual manner with 25 cc. of the hydroxide solutions in the dishes containing the pieces of agar. An examination of the auxograph record of the swelling thus obtained showed a remarkable similarity, in that after about 24 hours there was a marked acceleration in the rate of swelling, and the final results were very similar in all cases. In figs. 1 and 2 the curves of swelling are reproduced for potassium hydroxide and ammonium hydroxide. The total swellings thus obtained in the various solutions are given in table V.

TABLE V

SWELLING OF DRIED PLATES OF AGAR IN 25 CC. OF ALKALINE HYDROXIDE SOLUTIONS AT 15°C.; TOTAL SWELLING OF AGAR PLATES IN WATER 3950 PER CENT

Normal concentrations	Water	NH_4OH	LiOH	NaOH	KOH
0.01	100	63	60	55	49
0.001	100	77	77	75	81

Titrations after 24 hours of the solutions in which the agar had been swelling showed that the solutions had decreased

[3] BIRCKNER, V., Jour. Biochem. **38**:245–254. 1919.

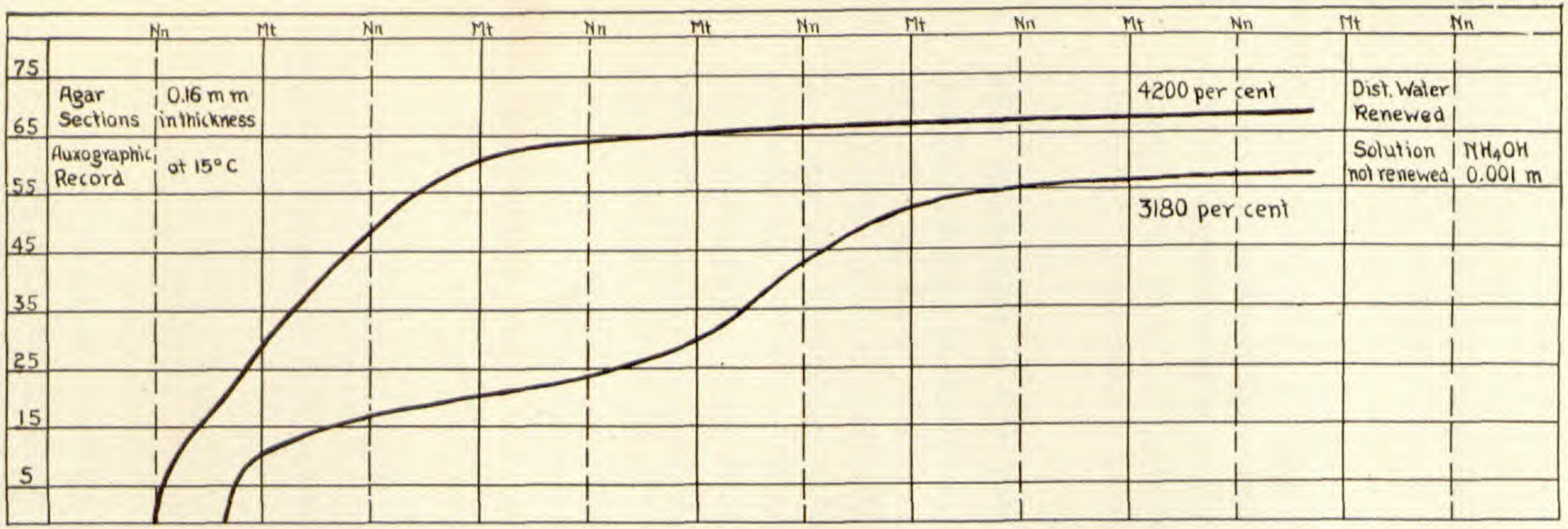

FIG. 1.—Auxographic record of swelling of agar plates in 0.001N solution of NH_4OH not renewed, and in water renewed every 24 hours

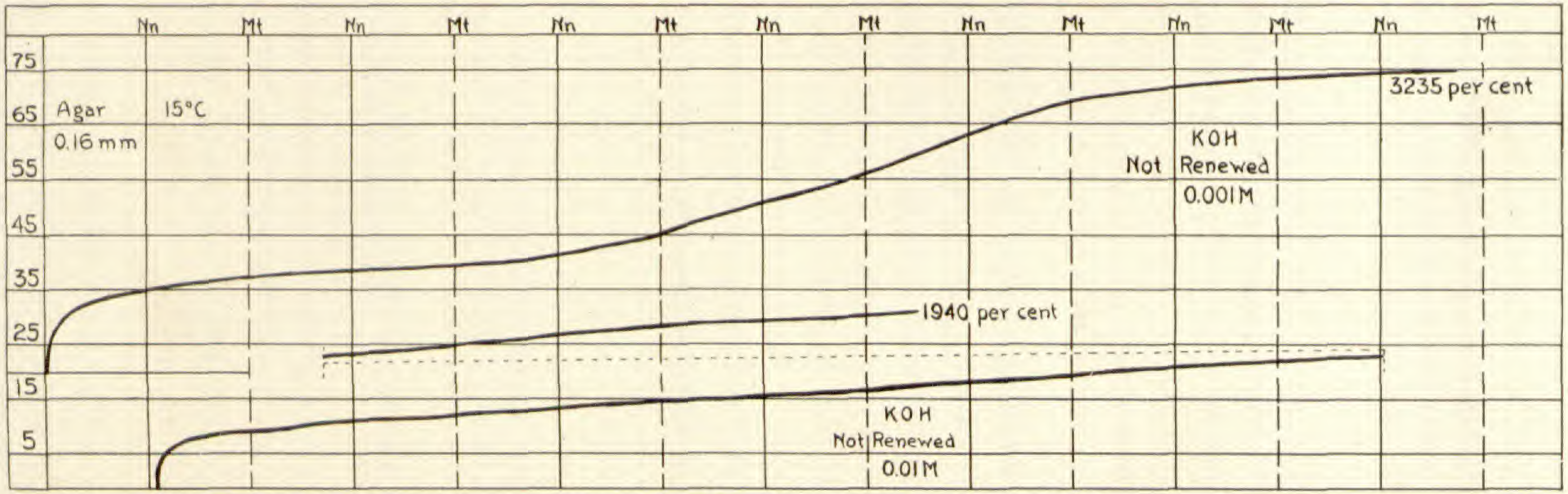

FIG. 2.—Auxographic records of swelling of agar plates in 0.01 and 0.001N solutions of KOH not renewed during course of experiment.

considerably in strength in this time, probably due to absorption of CO_2 from the air, and, in the case of the ammonium hydroxide, to volatilization. Experiments were then made in which the solutions were removed from the agar and replaced by fresh solutions every 12 or 24 hours. The results thus obtained differ radically from the previous ones, as to form of the record made by the swelling agar, as well as to the total amount of swelling attained in each case. Figs. 3 and 4 give the auxograph record of the swelling agar in solutions renewed every 12 hours. The relatively sudden increase in rate of swelling after 24 hours, which was so striking in the experiments in which the solutions were not renewed, is entirely absent. This accelerated swelling undoubtedly represents the rate of swelling in a solution which is but slightly alkaline and approaches that obtained in water. Furthermore, the total swelling of agar in KOH, NaOH, and LiOH is decidedly lower in the solutions which had been renewed. Especially noteworthy, however, is the fact that ammonium hydroxide in 0.001 normal concentration produces a swelling considerably in excess of water when the solution is renewed. This observation has been verified repeatedly.

The differences in the swelling of agar in NH_4OH and $C_2H_5NH_2$ on the one hand, and in LiOH and KOH on the other hand, are considerable, particularly in the more dilute solutions.

TABLE VI

SWELLING OF DRIED AGAR PLATES AT 15°C. IN ALKALINE HYDROXIDE SOLUTIONS, RENEWED EVERY 12 HOURS; TOTAL SWELLING OF DRIED AGAR PLATE IN WATER 3950 PER CENT

Normal concentration	Water	NH_4OH	Ethyl amine	LiOH	NaOH	KOH
0.01	100	25	31	24	21	21
0.001	100	115	88	40	35	29

Owing to the fact that in solutions of ammonium hydroxide and ethyl amine there exist equilibria respectively between dissolved NH_3 and the hydroxide, and between dissolved $C_2H_5NH_2$ and its hydroxide, the condition in solutions of these substances, particularly in the more concentrated solutions, offers a rather complicated situation not dissimilar from that obtaining in solutions

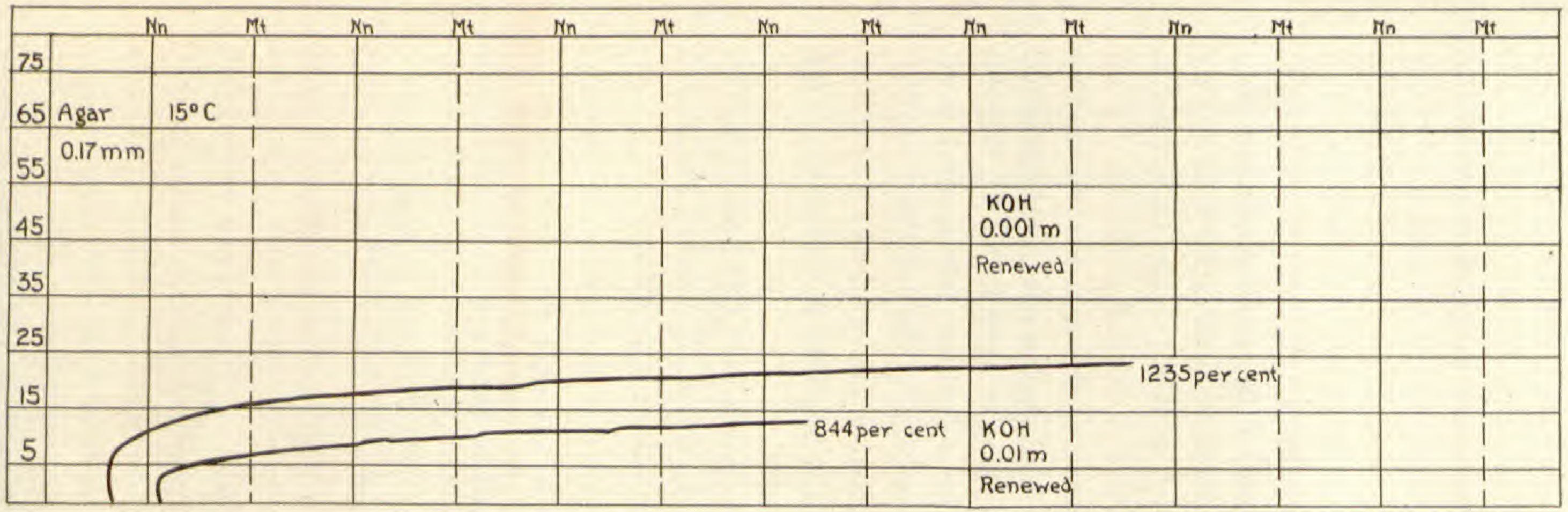

FIG. 3.—Auxographic records of swelling of agar plates in 0.01 and 0.001N solutions of KOH renewed every 12 hours

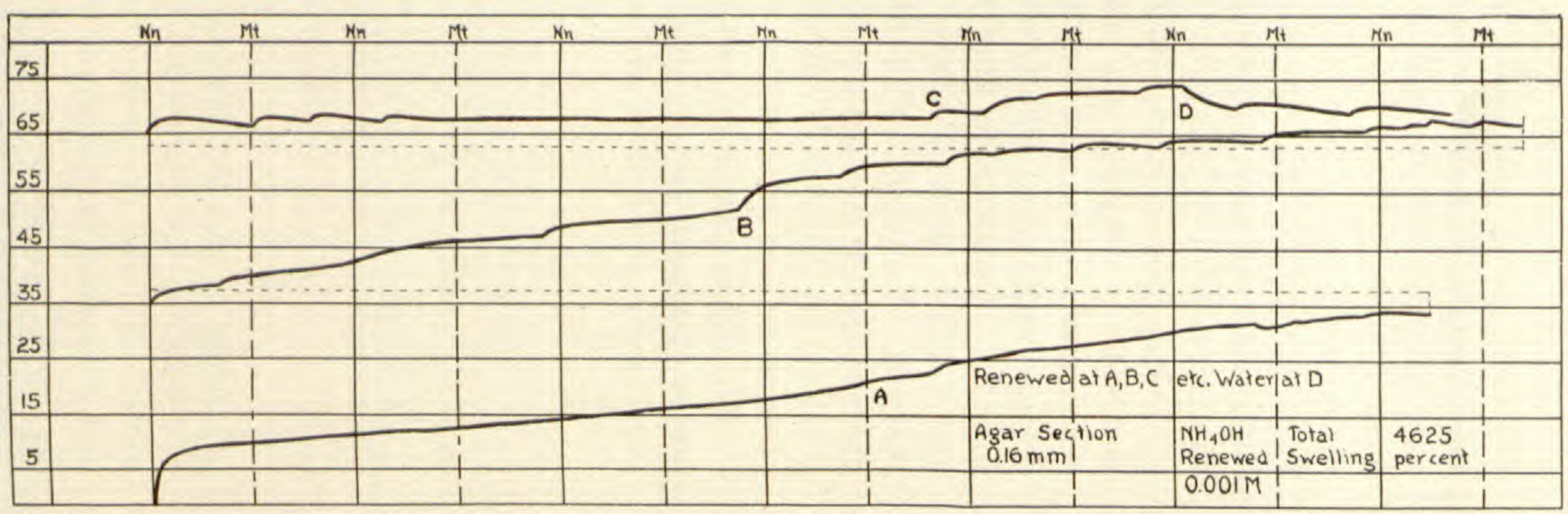

FIG. 4.—Auxographic record of swelling of agar plates in 0.001N solution of NH_4OH renewed frequently and replaced by water at *D*

of the amino acids. From these experiments it would appear that the stronger the base, as indicated by its position in the electromotive series, the less is the effect on the swelling of agar. Thus we have in effect K<Na<Li, and, in the weaker concentrations, ethyl amine, which is a stronger base than ammonium hydroxide, falling below this in hydration capacity. Interesting is the case of aniline, a very weak base. Separate experiments with this substance were carried out, in which agar showed the following swelling: in 0.01N solution, 110; in 0.001N solution, 100; in water, 100.

Further investigation of the effect of a possible agar-salt formation on the swelling was undertaken by a study of the behavior of dried agar plate which had been prepared from agar previously treated with KOH and NH_4OH. Portions of the purified agar were allowed to remain in such a quantity of 0.01 normal KOH and NH_4OH solutions as to make up a 2.5 per cent agar solution. After about 14 hours these solutions with the agar were heated, and the agar plates poured and then dried in the usual manner. The "kaliated" and "ammonated" plates thus prepared were then allowed to swell in solutions of KOH, NH_4OH, and distilled water. During the first 24 hours the water in which these plates had swelled became distinctly alkaline. Thereafter the water was renewed every 48 hours and showed but slight alkaline reaction. Thus the hydrogen ion concentration of the water in which the plates had been swelled, as determined by the indicator method, gave the values shown in table VII.

TABLE VII

Time	"Kaliated" plate P_H	"Ammonated" plate P_H
August 13.....	10.0	10.0
August 15.....	7.4	7.2
August 17.....	7.7	7.4

The figures given for August 15 and 17 probably indicate the values representing the products of hydrolysis of the "kaliated" and "ammonated" plates. The total swellings of these plates are given in table VIII.

From the results thus far obtained it would appear that the swelling of agar in water is surpassed only by that taking place

under conditions favorable to the formation of a combination of agar with ammonia or its closely related compounds. The exact nature of these agar combinations and the manner in which water acts upon them have not as yet been determined with any degree

TABLE VIII

SWELLING OF DRIED "KALIATED" AND "AMMONATED" AGAR PLATES AT 15°C.; ACTUAL SWELLING IN WATER OF "KALIATED" PLATE 3450 PER CENT; "AMMONATED" PLATE 2450 PER CENT

Plate	Water	0.01 N KOH	0.001 N KOH	0.01 N NH_4OH	0.001 N NH_4OH
"Kaliated"	100	57	83	.....	.....
"Ammonated"	100	.....	.....	107	122

of satisfaction. Ammonium salts, such as ammonium chloride and ammonium acetate, in the concentrations thus far tried, have shown no augmenting effect on the swelling. Ammonium acetate, although neutral in water solution, is hydrolyzed to a considerable extent with the formation of the ions of ammonium and acetic acid. The effect of these salts, in which the nitrogen can be considered as fully satisfied, on the swelling of agar shows a typical neutral salt effect.

TABLE IX

SWELLING OF DRIED PLATES OF AGAR AT 15°C. IN SOLUTIONS OF AMMONIUM CHLORIDE AND AMMONIUM ACETATE; TOTAL SWELLING OF AGAR PLATES IN WATER 1700 PER CENT

Normal concentration	Water	NH_4Cl	$NH_4CO_2CH_3$
0.01	100	58	58
0.001	100	94	100

An examination of the records produced by swelling agar in which the solutions are regularly renewed indicates clearly that the addition of a fresh solution of ammonium hydroxide affects distinctly the subsequent swelling of the already partially hydrated agar (figs. 3, 4). Thus, for example, sections of agar had swelled to values in water = 100; in 0.01N NH_4OH = 25. After the sections had attained their full swelling of 25 in 0.01N NH_4OH, the hydroxide solution was replaced by water. The agar swelled further to a value equal to 73. Thereupon other sections of dried

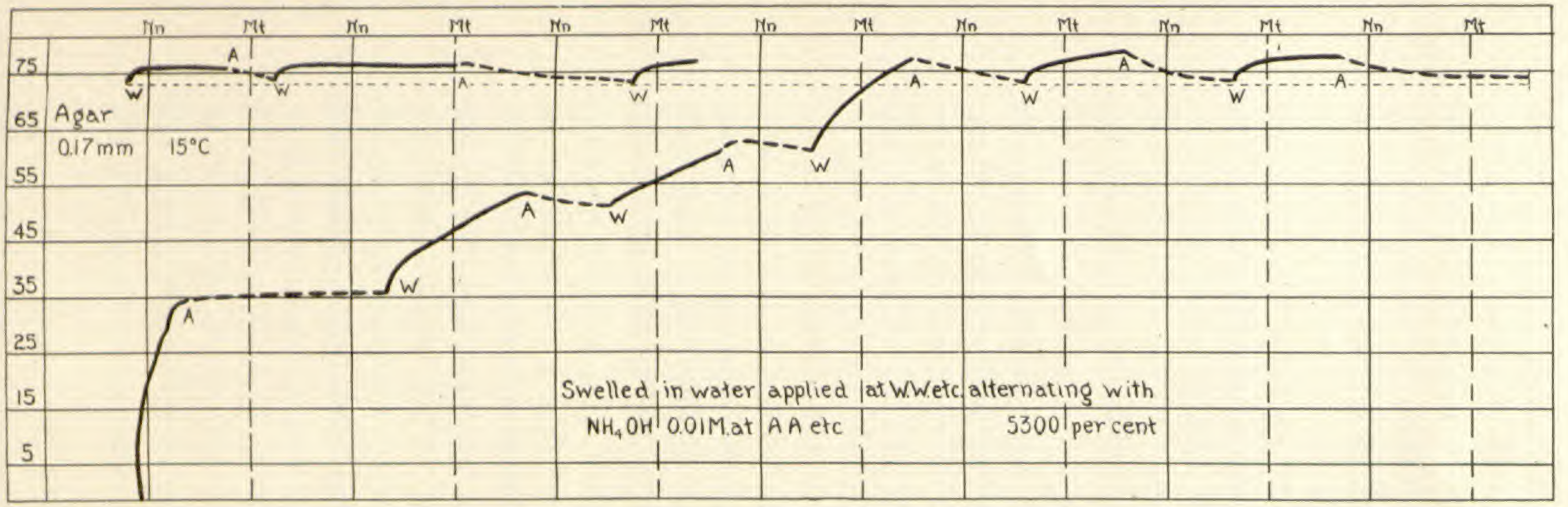

FIG. 5.—Auxographic record of swelling of agar plates in 0.01N solution of NH_4OH alternated every 24 hours with water; broken line indicating swelling in NH_4OH.

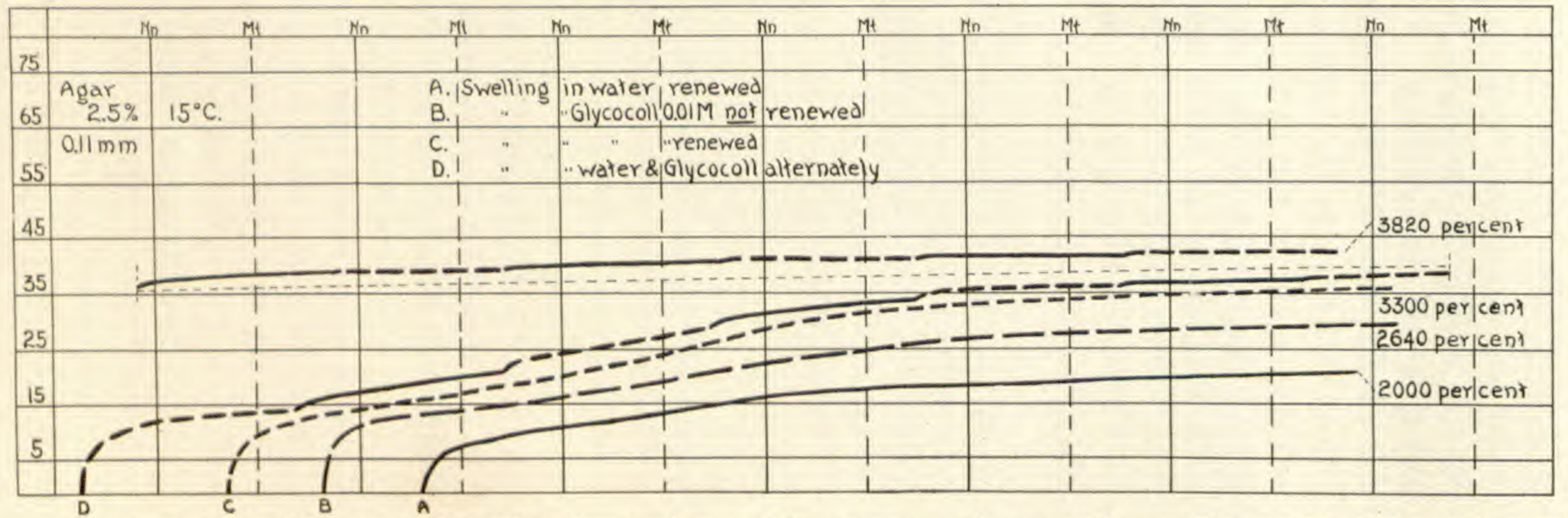

FIG. 6.—Auxographic records of swelling of agar plates: *A*, in water renewed every 12 hours; *B*, in 0.01N solution of glycocoll not renewed; *C*, in 0.01N solution of glycocoll renewed every 24 hours; *D*, water and 0.01N solution of glycocoll alternating; broken line indicating swelling in glycocoll.

agar plate were allowed to swell by alternating every 12 hours water and ammonium hydroxide in 0.01N solution. The curve for this swelling is reproduced in fig. 5. At first both the NH_4OH and the water cause swelling. As the hydration continues, however, the swelling in NH_4OH becomes slight, and finally there is an actual shrinkage in the hydroxide with, moreover, a subsequent swelling again in the water. The total swelling thus attained greatly exceeds that in water or ammonium hydroxide alone. The total swelling of the dried agar plates treated thus alternately with water and ammonium hydroxide was 5300 per cent, or on the basis of the swelling in water equal to 100, the swelling in alternating NH_4OH and H_2O equaled 134. Similar experiments were carried out in which glycocoll was alternated with water. Thus with 18 changes in 15 days agar swelled 191 as against 100 in water. In a solution of glycocoll which was not changed during the entire swelling the agar showed a swelling value of 132. The curves thus formed by the swelling of agar in glycocoll are shown in fig. 6.

No adequate explanation has been found for the augmenting effect on the swelling of agar produced by ammonia and the amino acids and here described. From a consideration of the relative effects of various hydroxides it would appear that something analogous to salt formation and subsequent hydrolysis of this compound may be involved.[4] Ammonium, as a weak base united with agar, an exceedingly weak acid, would form a salt which would very easily be hydrolyzed. Under conditions in which this hydrolysis is suppressed by the presence of a common ion (NH_4), the excessive swelling does not take place. Thus when dried agar plates are allowed to swell by alternating solutions of 0.001N, NH_4OH, and NH_4Cl the total swelling does not exceed that attained in water, but actually falls somewhat below that value.

The possible biological significance of these changes in volume, which are also exhibited by agar-protein mixtures with respect to growth and metabolism, seems to be so great as to warrant, not only this brief description, but also the formation of plans for the extension of the experiments.

DESERT LABORATORY
TUCSON, ARIZ.

[4] BRACEWELL, R. S., Jour. Amer. Chem. Soc. 41:1511–1515. 1919.

EARLY STEM ANATOMY OF TODEA BARBARA

JAMES E. CRIBBS

(WITH PLATES XXIII–XXVI)

The work of JEFFREY (6) and FAULL (4) on the Osmundaceae, in which they interpreted the stele as containing a medulla of cortical origin, has stimulated an investigation into the condition of the stele as found in the ancestry of the line, and has brought forth a great deal of criticism.

The present investigation of the early sporophyte stages of *Todea barbara* was undertaken to ascertain whether in the early periods of organization and development there might be some phases which would be significant as to the ancestral stelar condition. Spores were sown, and from the resultant gametophytes large numbers of sporophytes were secured and examined in all stages of development up to the time of departure of the fourteenth leaf trace. Considerable variation appears in the development of the young sporophyte and in many of its anatomical features. This is especially true of the young plant before it has established its independence of the gametophyte. The first root, for instance, sometimes elongates first, breaking through the old venter wall, but more commonly the leaf takes this initial step. There is irregularity in the phyllotaxy of the first leaves, in the appearance and attachment of the first roots, in the appearance of sclerenchyma, and in the time of medullation.

The earliest protoxylem to appear has been observed to specialize opposite the foot attachment, where it continues downward in one of the two protoxylem points of the root, and upward into the stem, where it is associated with the elements which are diverted into the first trace. Part of the protoxylem elements of the second group in the root turn out and enter the foot region, while the others are oblique and are continuous into the basal portion of the stem, or may terminate at the junction of the foot and stem,

forming triaxial tracheae which are in contact at the three points with the tissues of the three plant regions.

The young stem directly above the foot attachment contains xylem elements which are relatively short and grouped in a typical protostelic manner. They are surrounded by a single layer of nucleated parenchyma cells, external to which a few sieve tubes are disposed in a much broken circle. These are succeeded externally by a pericycle layer one cell in width, and which, like the phloem, is more or less discontinuous. Frequently an embayment occurs in the xylem at the edge of the foot attachment with the stele (fig. 6). This is occupied by parenchyma which is usually continuous with that of the root which lies between the protoxylem points, and extends up into the stem, where it usually becomes more shallow, and is exserted before the departure of the first leaf or soon thereafter. This indention, when present, occurs at right angles to the plane of the foot attachment and never in the position of a gap (fig. 32).

The first leaf trace is detached from the stele about 70–150 μ above the foot, and is preceded by the appearance of 1–3 parenchyma cells in the xylem, which assume an eccentric position beneath the place of leaf exit. Quite commonly these first cells are in contact laterally with the sheath parenchyma; but whether in contact below or not, they become confluent with the sheath parenchyma at the time of exit of the first trace, when the xylem of the stele once more forms a solid group. It is quite evident, therefore, that this first xylem parenchyma is merely an accompanying feature of the departure of a simple trace from the protostele. Very similar to the preceding is an unusual behavior observed when an embayment of parenchyma at the foot attachment became decurrent in the xylem of the root. This parenchyma cell could not be shown to be in contact with that surrounding the xylem, but apparently was lost in the midst of the metaxylem elements of the root, just as the decurrent parenchyma associated with the departure of the early traces in the stem may end blindly in the tracheae a short distance below.

DeBary (3) considered the stele of the Osmundaceae as a sympodium of leaf traces, and, in reference to the young stem,

states: "The first bundle, which usually ends blind in the foot of the embryo, curves after a very short course through the stem into the first leaf; from the point of curvature the development of a bundle, which runs out into the second leaf, begins. In the case of the subsequent leaves the same conditions prevail." DEBARY'S interpretation of the stelar structures in this group has been supplanted by the theory of the stele as a unit in stem development (VAN TIEGHEM 12), which was strongly supported by GWYNNE-VAUGHAN'S (5) work on *Primula*, and subsequently by others, and which has at present gained most general credence. The conditions found in the young stem of *Todea barbara* give evidence in support of the later theory; for, as is readily observed, the trachea group above the foot is composed of 15–25 elements, while the number entering the first leaf is commonly but four, and was never observed to exceed six (fig. 5).

The stem axis bends at each node in the young sporophyte, so that the traces depart from the outer angles, a behavior undoubtedly referable to the manner of origin of the leaves, and is caused by pressure of segments cut off from the leaf apical cells and stem apical cells following the isolation of the former in a small meristem which has not yet become closely invested with leaf bases (figs. 35, 37). In the early organization of the stem a single apical cell appears, which is usually of the three-sided pyramidal type. Sometimes it is truncated at the base, and in most instances was found to be broader near the middle than at the top (fig. 36). Frequently, however, the apical cell is four-sided in transverse section (figs. 39, 41), and, like that of the root, cuts off segments more or less irregularly, often giving the appearance of a cluster of initials (figs. 38, 46). Only one instance of initials not certainly referable to a single cell was found (fig. 40). This instance occurred after the organization of the medulla, and appears to be a true case of more than one apical cell; but the earliest meristems were always referable to a single initial. At the level of departure of the first trace there is still a paucity of sieve cells (fig. 5); the pericycle is likewise incompletely developed, and, in fact, the feeble development of all the extra-xylar elements is quite noticeable in contrast with their relative prominence at higher levels in the

stem. The trachea elements of the early stele are strikingly uniform in caliber, pointed at the ends, relatively short, and display no certain delimitation of protoxylem and metaxylem.

Above the point of separation of the first and subsequent leaf traces a groove commonly occurs in the xylem axis directly opposite the departing strand (figs. 7, 9, 11, 15). This is continuous upward a variable distance, where it gradually becomes more shallow and disappears. Not infrequently, however, after the first one or two traces have departed, this groove may detach or be connected internally with a single parenchyma cell which passes up in the center of the trachea group, where it occasionally ends blindly, but more commonly becomes continuous with the sheath parenchyma once more through the embayment of decurrent parenchyma associated with the departure of the succeeding trace. Frequently such central parenchyma cells are in continuity externally through lateral embayments at the edge of root attachments, similar to that which occasionally accompanies the attachment of the foot. A leaf trace sometimes departs from the stele, which includes parenchyma decurrent from the trace above, without being accompanied by a break in the xylem cylinder.

In fig. 8 two included parenchyma cells appear, which are in contact with the sheath above (fig. 9). The embayment occurring opposite the departure of the second trace in this instance does not close up, but becomes continuous with that which isolates the third trace (figs. 10, 11). Above the third trace the protostele becomes divided in the plane of the exit of the last trace (fig. 12), but, contrary to expectation, neither segment is exserted as a trace, but the succeeding trace is detached from the segment to the right at a higher level (fig. 13). The two remaining strands fuse almost immediately, and subsequently an indention occurs opposite the last trace (figs. 14, 15). This is continuous with a group of two parenchyma cells which becomes centrally located (fig. 16), and is in contact with the sheath parenchyma again at the departure of the fifth trace (fig. 17). Reference to this series shows that five leaves take their departure from the stem before the organization of the second root, which appears directly beneath and is associated with the sixth leaf (fig. 18). The earliest phyllotaxy

in this instance is expressed by the fraction $\frac{2}{5}$. It later changes to a $\frac{3}{8}$ arrangement, which appeared most frequently in the early stem.

A solid protostele occurs a short distance above the fifth trace, and is illustrative of a constant recurrence of a perfectly solid stele associated with the departure of either the fifth or sixth foliar strand (fig. 18). The appearance of the protostele at the detachment of the fifth trace is shown in fig. 28, which is typical of this level of the stem in all of the young plants studied. It will be noted that the xylem is composed of tracheae alone, although internal parenchyma was present both above and below the three preceding nodes, and is constantly present above the level of the sixth trace. Although the occurrence of internodal parenchyma is common in the young stele, the number of elements is usually very limited. Occasionally, however, the internodal pocket becomes quite extensive (fig. 1), when the central cylinder assumes the appearance of a true siphonostele with centrally placed thin-walled elements. Yet despite the siphonostelic aspect, the stem becomes distinctly protostelic at the node above, and also at the level of exit of the fifth and sixth traces. The parenchyma pocket shown in fig. 16 is quite the normal condition, and is seen to be a basipetal extension from the cleavage of the stele at the node above.

It has already been pointed out that the departure of a trace from the protostele may so influence the xylem that it becomes entirely divided into two segments. A recurrence of this is shown in fig. 19, in which instance, however, the indentions of both the fifth and sixth traces are concerned, affecting the stele from opposite sides. The two residual strands coalesce immediately above the node as in the former instance cited (fig. 12). The eighth trace takes its departure from the stele in a similar manner to the fifth, leaving a solitary intruded parenchyma cell which is soon replaced above by tracheae, leaving a solid protostele (fig. 20). Even at nodal regions the stele was always observed to contain thin-walled elements above the level of the eighth leaf, while more commonly the fifth or sixth node marks the upper limit of a stele entirely free from these elements.

There is a gradual increase in the number of xylem elements accompanying the enlargement of the stem up to the time of

medullation (figs. 22, 23). In the change from the protostele to a siphonostele there is a rapid increase in the diameter of the stem, and an appearance of parenchyma cells with included resinous storage similar to those of the pericycle. These increase rapidly in number with the appearance of a permanent medulla, but so far as observed no real sclerenchyma of the type found in the adult stele occurred in young stems up to the time of the separation of the fourteenth leaf trace (fig. 24). This early organization of the medulla takes place without the appearance of internal phloem, nor could sieve cells be demonstrated at any level in the central parenchymatous elements of the young stele. It was further observed that there was no endodermal invagination accompanying medullation, or the departure of traces from the young stele, up to that time. True leaf gaps occur in the stele after the appearance of the medulla, and the traces take their departure in a manner already described by SINNOT (11).

The storage parenchyma cells already mentioned as being very prominent in the early medulla have been observed to occur as early as the time of separation of the third trace, but more commonly about the time the sixth trace leaves the central cylinder. The first elements of this character are usually located opposite the place of exit of a trace and appear free in the sheath parenchyma (fig. 2), or occur at the edge of a root attachment. In a few instances they have proved to be continuous with like elements of the pericycle layer, the storage contents of which cells they resemble. The most pronounced intrusion of such cells observed in any of the young stems is shown in fig. 44, where they occur opposite a trace and at the edge of a root attachment. In no instance, however, has it been possible to demonstrate any thickenings on the walls of these elements such as occur on the endodermis, and it seems that their relation to the pericycle is not at all constant.

The endodermis is organized early in the development of the sporophyte, and is continuous over the early stem, the primary root, and the tissues which elongate and diverge into the foot. At a comparatively early stage it closes over the conductive elements in the foot, and thus entirely incloses the stele from the root apices to the meristematic region of the stem apex. A transverse section

of the foot in a three-leaved sporophyte is shown in fig. 26, and it will be noticed that it almost caps the foot tissues at this early stage, only two cells remaining free. Throughout all the stages, so far as studied in the young stem, there was no indication whatever of the endodermis dipping into the stele; but in complete continuity it passes over the gaps in the xylem caused by the departing leaf traces, without the slightest tendency to invaginate. A commonly recurring feature of the endodermis from about the level of the sixth leaf trace was the absence of storage materials opposite leaf gaps. Frequently there was likewise an absence of storage in the pericycle at the same point. Across this gap in the storage material, however, the endodermis was always found to be continuous.

The origin of the pericycle from the stem apex is difficult to determine, and it could not be definitely referred to initials which would point to a common origin with the endodermis. For the most part the pericycle cells contain an abundance of finely granular resinous material. As has already been pointed out, the pericycle does not form a complete circle in the earliest stages of the stele, but is interrupted at many points. At the level of the sixth or eighth trace it is rarely more than one cell thick, and in the young sporophyte it seldom exceeds two cells in thickness except at the edge of root attachments and in the adaxial angle of the leaf traces, where it frequently becomes very prominent (figs. 3, 44), and, like the endodermis, is quite regularly filled with finely granular material or with larger granules similar to those of the storage cells in the sheath parenchyma and medulla. The pericycle is increased in thickness by periclinal divisions (fig. 1), but the so-called "quergestrickten Zellen" of the older stem which have their origin in this way do not appear prominently in the earliest stages, but appear much more abundantly after the development of a central medulla.

There is a paucity of sieve cells in the lower levels of the stele, where they are most prominently and regularly developed on the outer edge of the xylem elements which are about to turn out from the cylinder as traces. The sieve cells are elongated elements terminated by oblique walls. The radial, terminal, and likewise the

tangential walls when in contact with similar cells are perforated with openings which vary from simple pits to relatively large sieve plates with numerous small apertures. The transversely elongated elements derived from the pericycle by tangential divisions are, as pointed out by SEWARD and FORD (10), sieve elements which are of later origin than the sieve tubes of the protophloem. The sieve plates of these elements, as asserted by FAULL (4), show callus plugs, and all the essential features of true sieve cells are present. There was no evidence of the appearance of phloem in the internal parenchyma in any of the material investigated, nor were the sieve cells ever observed to appear in the parenchyma tissue of the foliar gaps.

As already stated, there is a single row of nucleated parenchyma cells which surround the xylem in the first stages of the stele. At higher levels these elements increase in amount by tangential divisions. In longitudinal view they are observed to be narrow, elongated, with acutely oblique terminal walls. These resemble very closely the earliest xylem parenchyma. Are these elements to be considered as of cortical origin, as asserted by JEFFREY (7) and FAULL (4), or are they to be considered as distinctly stelar and representing undeveloped potential xylem elements differentiated by the meristematic stem apex? In the young stele these cells do not resemble the cortical tissues either in topography or cytology. The central ones at first have distinctly acute terminal walls, and in general topography are altogether like the tracheae, except, of course, for the secondary thickenings of their walls. At higher levels these internal unthickened elements frequently have transverse terminal walls or are but slightly obliqued from the horizontal. Further evidence for the stelar origin of these elements is found in the occurrence of tracheids in the same linear series with parenchyma cells above and below (fig. 29). It was further noticed in such instances that the tracheids were terminated by walls which were transverse or almost so, instead of having the strongly oblique prosenchymatous type so characteristic of the normal tracheae of the xylem axis. Cells of this type were found on the inner edge of the xylem in contact with the parenchyma, and in a few instances on the outer border where

they were in contact with the sheath parenchyma. A first supposition that these were protoxylem elements was readily disproved; for the cells are continuous up the stem axis, and in no instance turned out into the diverging xylem group of the leaf traces, as do the protoxylem cells. Their squared terminal walls are likewise not characteristic of protoxylem as found in the stele and traces. They always were observed to include some cytoplasm and most frequently a degenerate nucleus. A transverse view of these cells is shown in fig. 27, where it will be noted that they have a thickening equal to that of the normal tracheae, and in conjunction with the true xylem elements do not develop the lacunae between cell walls except occasionally, when it is barely distinguishable. They also lack the angle thickenings so characteristic of the Osmundaceae. In view of their belated appearance, their topographical and cytological features, they are considered as abnormal tracheae, arrested in development, and are considered as evidence indicative of stelar origin of the medulla by reduction. SEWARD and FORD (10) referred to similar elements found in *T. superba* and *T. hymenophylloides*, and KIDSTON and GWYNNE-VAUGHAN (8) interpreted them as probably being vestigial. That these are the last remnants in our living species of the peculiar central xylem now known to have been present in *Kalesskya* (8) and *Thamnopteris* (9), extinct protostelic members of this family belonging to the Upper Permian, seems entirely probable, and is the most logical interpretation.

The primary root of the sporophyte, as well as the roots which appear successively with the development of leaves in the early plants, are characteristically diarch (occasionally triarch), with radially arranged phloem. A narrow zone of parenchyma separates the xylem and phloem in a manner similar to that in the young stem stele (fig. 43). Following the development of the first root, two or more leaves regularly occur before the appearance of the second. This in most instances is associated with the third or fourth leaf, but in a few cases it has been observed to appear considerably later. In the series represented by figs. 6–24 it will be noted that the second root is associated with the sixth leaf. Roots, following their delayed appearance, frequently develop in an aberrant manner, forming often without any particular

relation to departing traces. Leaves frequently develop without corresponding roots. This early vacillating condition is replaced by regularity after the establishment of a central parenchyma, and a single root takes its departure from the stele just below the attachment of the leaf trace. Subsequently there is a second period of irregularity when either one or two roots are detached from the stele with each leaf.

In his studies of the apical regions of *Todea barbara*, BOWER (1) states: "The roots take their origin from a single cell of the endodermis which is situated opposite a xylem strand." In all cases observed in the young stem the root apical cell originated in the pericycle (figs. 45, 48). The initial cell enlarges, and, following a few radial divisions, a single apical cell develops which is quite variable in shape, but very early organizes into a three-sided pyramidal cell (fig. 47). BOWER (1) further states that of a number of root apices examined in *Todea barbara* "not one showed a clearly marked single apical cell. Some, however, showed somewhat irregular arrangements, and in some it appeared uncertain whether the meristem be referable to three or four initial cells. In the majority of the roots observed it is clearly referable to four initial cells, separated from each other by the four principal walls." In examining the root meristems in the young plants of *Todea barbara*, 28 single apical initials were recorded in as many different plants, and not a single instance of four initials was observed. There were a few cases which showed some variation from the pyramidal three-sided cell, with the divisions occurring unevenly. The segments from the apical cell are usually very large and do not divide immediately, resulting in a meristematic group resembling in longitudinal view a cluster of three or four initials. These, however, have always been referable in the root to a single initial which was prevailingly of the triangular-pyramidal variety. Thus the coaxial type described by BOWER (1) was not found in any of the root meristems of the early sporophytes.

The outer cortical cells of the primary root are usually the first to develop the sclerenchyma thickenings which later become so conspicuous a feature of the stem cortex. From the primary root they are extended to the adjoining cells of the stem in which

they persist throughout the axis. Frequently, however, the lower portion of the stem cortex is free from sclerenchyma, in which instance it is found to appear in the stem at the place of attachment of the second root, from the base of which it extends about the stele. When it first appears in this manner, the petioles of the leaves below the point of introduction most generally lack the characteristic cortical wall thickening of the cells surrounding the central bundle. After its first appearance in the stem it is sparingly developed or absent from the base of the next one or two petioles, although quite abundant in the stem axis and the leaves at higher levels, which would indicate that the rachis has been the last region of the plant to develop the sclerenchyma. An endophytic fungus was found to occur frequently in the cortical tissues of the root, external to the endodermis and internal to the sclerenchymatous cells of the peripheral region. It was found to gain entrance by way of root hairs, and also by dissolving its way through the epidermal cell wall at the edge of the root cap (fig. 42).

The first leaf originates at a point about 0.1 mm. above the foot attachment. The trace is isolated by an embayment, and carries off from the main axis about four cells (varies from three to six), which usually form a narrow band (fig. 5) which, once in the petiole, usually becomes endarch in its arrangement (fig. 33). Subsequent traces depart in a similar manner, carrying out an increasingly greater number of xylem elements until the appearance of a definite medulla tissue. The first petioles are practically wingless, but with an increase in leaf size and number they overlap and become conspicuously winged. The metaxylem elements meanwhile become more numerous, spreading out so as to form a semicircle with the opening toward the stele (fig. 34). The poorly defined phloem of the first trace becomes more definite and abundant in the second and third, where it may form a complete circle about the xylem.

In the petioles of the lower leaves the xylem sometimes assumes a distinctly mesarch arrangement, but is not completely invested by phloem (figs. 4, 25). The occurrence of primitive structures in the basal petioles has long been recognized, and the appearance of mesarch strands here is further evidence for the origin of our

present Osmundaceae from a protostelic group. It becomes particularly significant when we observe that *Kalesskya* and *Thamnopteris* were characterized by traces which departed in a protostelic manner and were strongly mesarch until they had entered for some distance into the petioles, where the xylem opened out and presented an appearance like that in the rachis of *Osmunda* and *Todea*. As already stated, these early representatives of the Osmundaceae were present in the Upper Permian and much preceded the Lower Cretaceous siphonostelic species *Osmundites skidegatensis* to which JEFFREY (7) referred for the most primitive type of osmundaceous stele available. The recurrence of mesarch strands, abnormal central tracheids, and a typical protostele in the young stem of *Todea* are interpreted as indicative of the descent of our present Osmundaceae from a protostelic ancestry.

Summary

1. The young sporophyte of *Todea barbara* is protostelic.

2. Preceding the departure of the early traces one or more parenchyma cells appear in the xylem group, or occupy a groove which is in contact with the sheath parenchyma. These, at the time of isolation of the trace from the central cylinder, become confluent with the xylem sheath, leaving the stele solid, or grooved opposite the strand. This depression is continuous up the stem, where it either becomes more shallow and is lost, or detaches one or more parenchyma elements which become centrally placed in the xylem group where they end blindly, or are in continuity with parenchyma decurrent from the succeeding trace.

3. The stem meristematic tissue is derived from a single apical cell of the triangular-pyramidal type. It, like the root initial, shows variation in the order of segmentation. Only one instance not certainly referable to a single initial was found.

4. Roots originate from the pericycle in the young sporophyte and very early develop a single apical cell which is broadly triangular and pyramidal, characterized by variation in shape and order of segmentation. Segments are large and frequently give the appearance of two or more apical initials. All root meristems examined, however, were referable to a single initial.

5. The cortical sclerenchyma is apparently of root origin, and is extended to the stem cortex from the primary root or from those which develop later in association with leaves.

6. An endophytic fungus is frequently found in the root cortex. It was observed to gain entrance through root hairs and through newly formed epidermis at the edge of the root cap.

7. A phyllotaxy represented by the fraction $\frac{3}{8}$ is most frequently found in the young plant. A $\frac{2}{5}$ arrangement was recorded in addition to a few cases of irregularity.

8. Leaf traces usually are endarch while in the stem cortex, and the protoxylem elements, which at first form a narrow band, spread out on the adaxial embayment which occurs in the metaxylem higher up in the petiole of the leaves.

9. Typical mesarch bundles have been observed in the petioles of early leaves. These are interpreted as being indicative of the ancestral condition, and in fact present the type of arrangement found by KIDSTON and GWYNNE-VAUGHAN to be characteristic of *Kalesskya* and *Thamnopteris*, Upper Permian representatives of this family.

10. The transversely elongated phloem elements are derived from periclinal divisions of the pericycle, and, as recorded by SEWARD and FORD, are sieve tubes which, in the organization of the stele, appear considerably later than the protophloem.

11. No instance of phloem within the medulla or central parenchyma was found at any level in the young stele.

12. The endodermis is continuous over the primary root, foot, and early stem. At no place in the young stem does it turn in through the gaps or embayments in the xylem.

13. Internal endodermis could not be demonstrated at any stage in the young stele.

14. The earliest parenchyma to appear within the xylem is composed of elements with pointed ends, whose caliber and length are very similar to the xylem elements, and conspicuously unlike the cortical cells.

15. No sclerenchyma was observed in the medulla up to the time of departure of the fourteenth leaf trace, although parenchyma cells with included resinous substances were quite abundant.

16. After the first organization of a definite medulla, short, thickened xylem cells have been observed to be present on the inner edge adjoining the pith. These elements, although thickened, were poorly lignified, contained some protoplasm and a degenerate nucleus, had transverse or slightly obliqued terminal walls, were usually in axial continuity above or below with parenchyma elements, and when in contact with adjoining tracheae did not develop the intercellular lacunae so characteristic of the protoxylem and metaxylem of this family. These elements are considered as belated xylem of vestigial character, and, because of their position and relation to the internal parenchyma, are evidence of medullary origin by stelar reduction. Cells of this type were not found in either the petiole or root.

17. The appearance of mesarch traces in basal leaves, a protostele in the early stem, the similarity in topography of early xylem parenchyma to xylem, the appearance on the inner border of the xylem of peculiar short tracheids with transverse terminal walls, the entire absence of internal phloem, the absence of internal endodermis, and the complete unity of the endodermis (which shows no indication of invagination) are all features in the ontogeny of *Todea barbara* which are indicative of a protostelic ancestry. These features assume a special significance when we can correlate them with similar structures in primitive forms, and their recurrence in *Kalesskya* and *Thamnopteris* further validates the theory of the protostelic origin of our living Osmundaceae.

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EXPLANATION OF PLATES XXIII–XXVI

PLATE XXIII

FIG. 1.—Transverse section of stele between third and fourth traces, showing extensive parenchyma pocket and periclinal divisions of pericycle; ×300.

FIG. 2.—Stele with tenth trace and seventh root; storage parenchyma in xylem sheath opposite trace, and three xylem parenchyma cells; ×88.

FIG. 3.—Transverse section of stele and seventh root; absence of storage in endodermis and pericycle opposite trace; gaps of sixth and seventh leaves; extensive pericycle development opposite sixth trace; ×138.

FIG. 4.—Mesarch grouping of xylem elements in petiole of third leaf, $\frac{1}{3}$ mm. above separation from stele; ×118.

FIG. 5.—Transverse section of stele and first trace, showing early phloem cells and paucity of extra-xylar elements in stelar cylinder; ×58.

PLATE XXIV

FIGS. 6–24 inclusive, ×40 diameter.

FIG. 6.—Foot attachment and lateral embayment of parenchyma.

FIG. 7.—First trace and grooving of xylem opposite.

FIG. 8.—Xylem with two parenchyma elements included.

FIG. 9.—Separation of second trace and grooving of xylem.

FIGS. 10, 11.—Separation of third trace from stele.

FIG. 12.—Cleavage of protostele into two strands.

FIG. 13.—Fourth trace being isolated from strand to right.

FIGS. 14–16.—Embayment and inclosure of parenchyma elements preliminary to detachment of fifth trace.

FIG. 17.—Connection of parenchyma of preceding figure with that of xylem sheath at time of separation of fifth trace.

FIG. 18.—Solid protostele above fifth trace; first root detached immediately below this level at lower left.

FIG. 19.—Second cleavage of protostele, with seventh trace to right.

FIG. 20.—Solid protostele with detached tracheae, decurrent from root which leaves stele immediately above.

FIG. 21.—Enlarged xylem group just before medullation.

FIG. 22.—Early medullation and inclusion of first storage parenchyma cells; eleventh trace.

FIG. 23.—Twelfth trace and eighth root.

FIG. 24.—Increase in storage parenchyma cells and medulla; ninth root.

PLATE XXV

FIG. 25.—Mesarch bundle in petiole of first leaf; ×625.

FIG. 26.—Transverse section of foot, showing closing of endodermis in three-leaved stage; ×88.

FIG. 27.—Transverse section of abnormal tracheids; ×625.

FIG. 28.—Stele at time of departure of fifth trace, showing solid xylem group and prominent sheath parenchyma; ×300.

FIG. 29.—Longitudinal section of tracheid in axial continuity with xylem parenchyma cells; ×300.

FIGS. 30, 31.—Sieve tubes with oblique terminal walls; ×300.

FIG. 32.—Stele immediately above foot; lateral embayment to right of foot attachment and indention of stele to isolate first trace at left; ×300.

FIG. 33.—Normal endarch bundle of first trace; ×88.

FIG. 34.—Fifth leaf strand 2.25 mm. above origin; phloem encircles xylem which shows adaxial embayment; ×300.

PLATE XXVI

FIG. 35.—Stem apical cell with leaf initial isolated at left; ×300.

FIG. 36.—Longitudinal view of stem apical cell; ×575.

FIG. 37.—Isolation of leaf initial from stem apical cell; ×575.

FIGS. 38, 39.—Irregularity in segmentation of stem apical cell; ×575.

FIG. 40.—Abnormal meristem with apparently four initials; ×300.

FIG. 41.—Transverse view of normal stem apical cell; ×129.

FIG. 42.—Cortical cell of root with endophytic fungus; ×625.

FIG. 43.—Transverse section of normal root, showing diarch character; ×88.

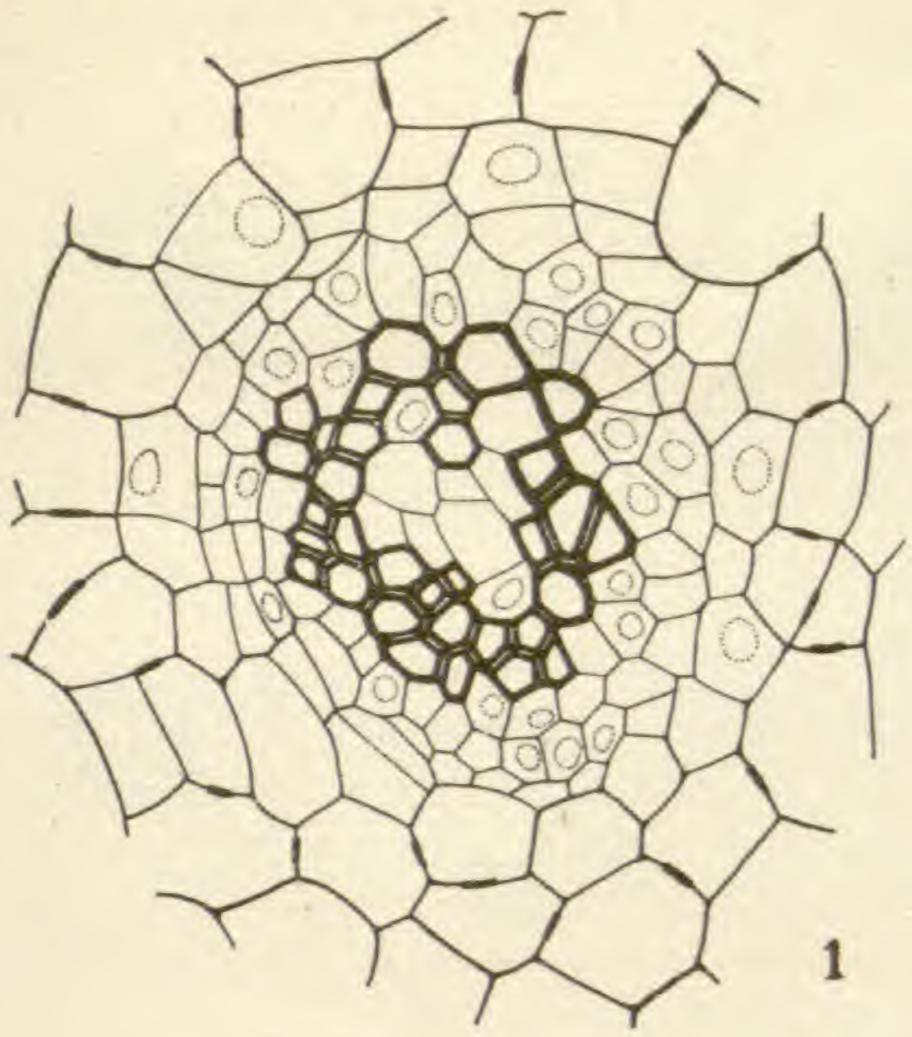
FIG. 44.—Transverse section of stem, showing at left a pericyclic intrusion and strong development at edge of root attachment; ×88.

FIG. 45.—Longitudinal section of root apical initial in pericycle; ×300.

FIG. 46.—Irregular segmentation of root apical cell; ×575.

FIG. 47.—Transverse section of triangular-pyramidal root apical cell; ×300.

FIG. 48.—Tangential section of stele, showing organization of root apex in pericycle; ×300.



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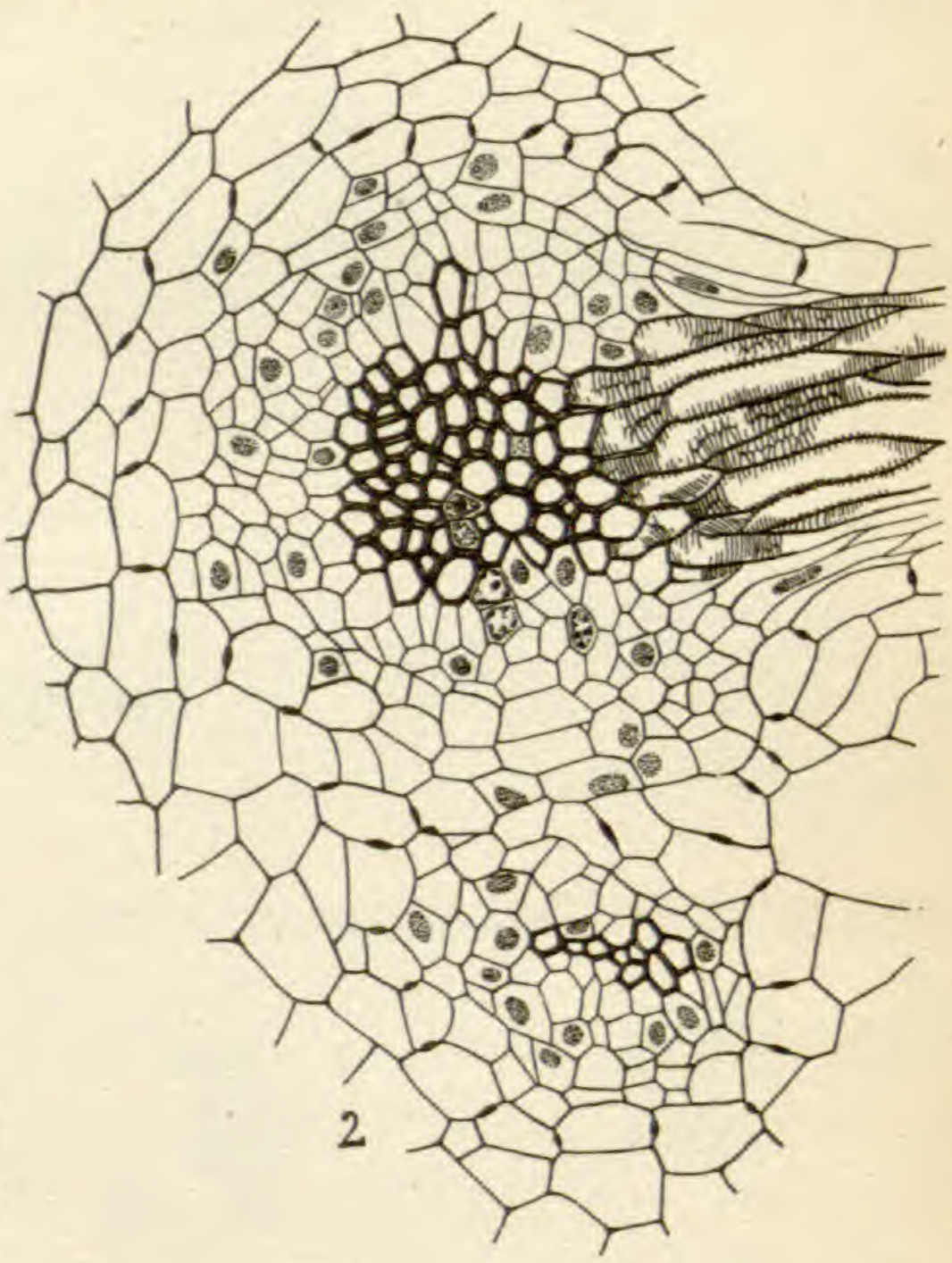

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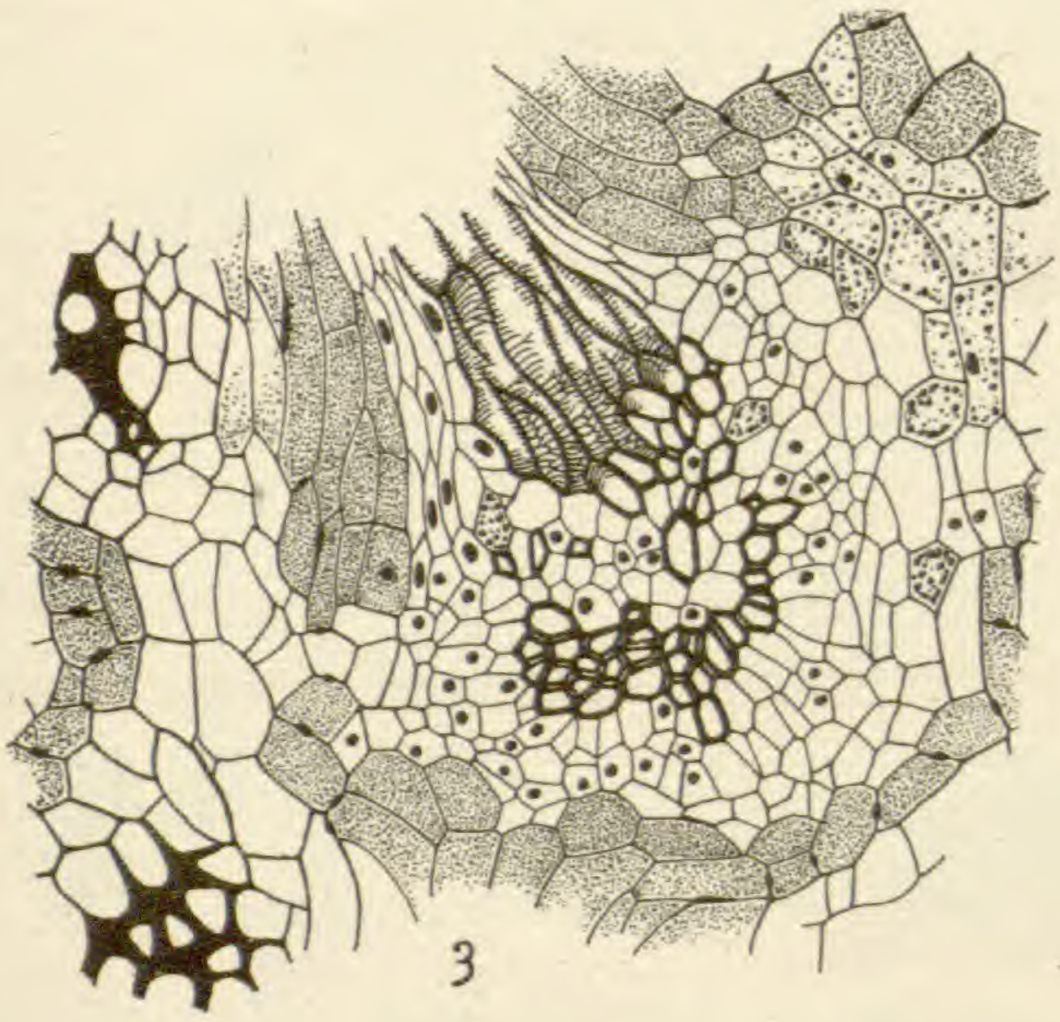

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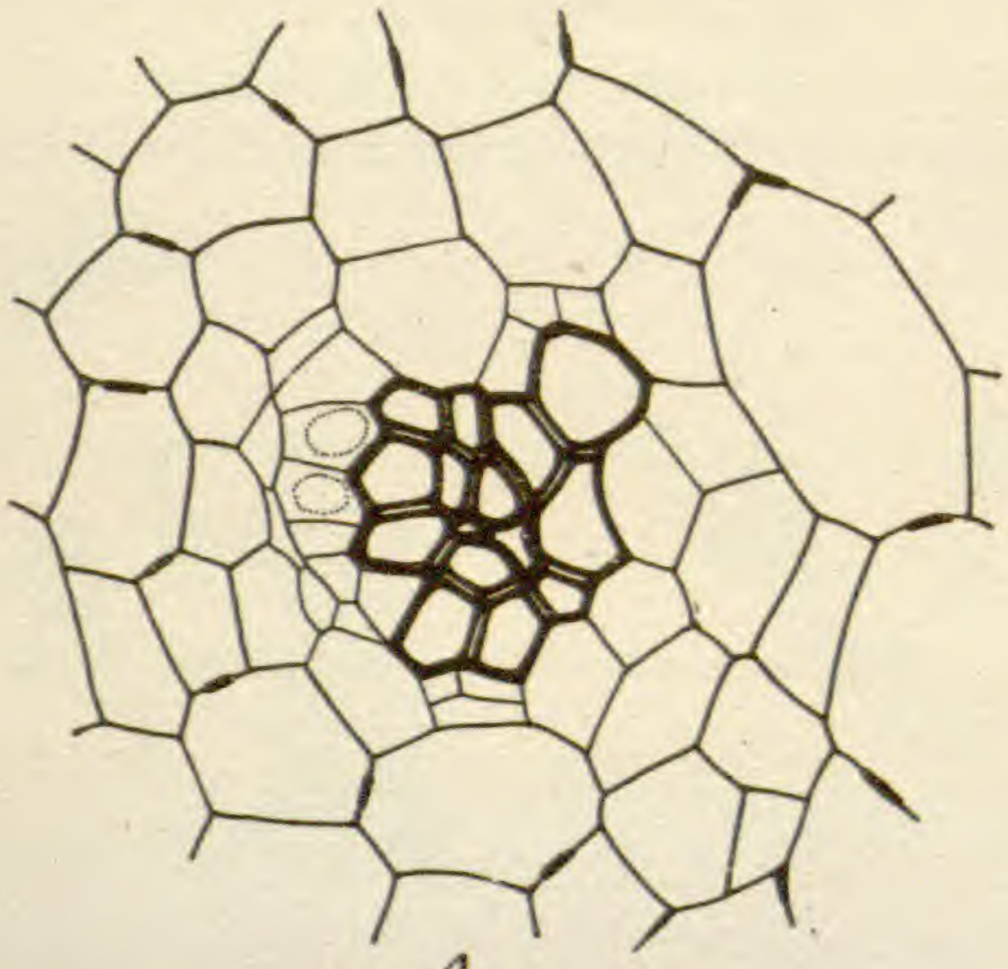

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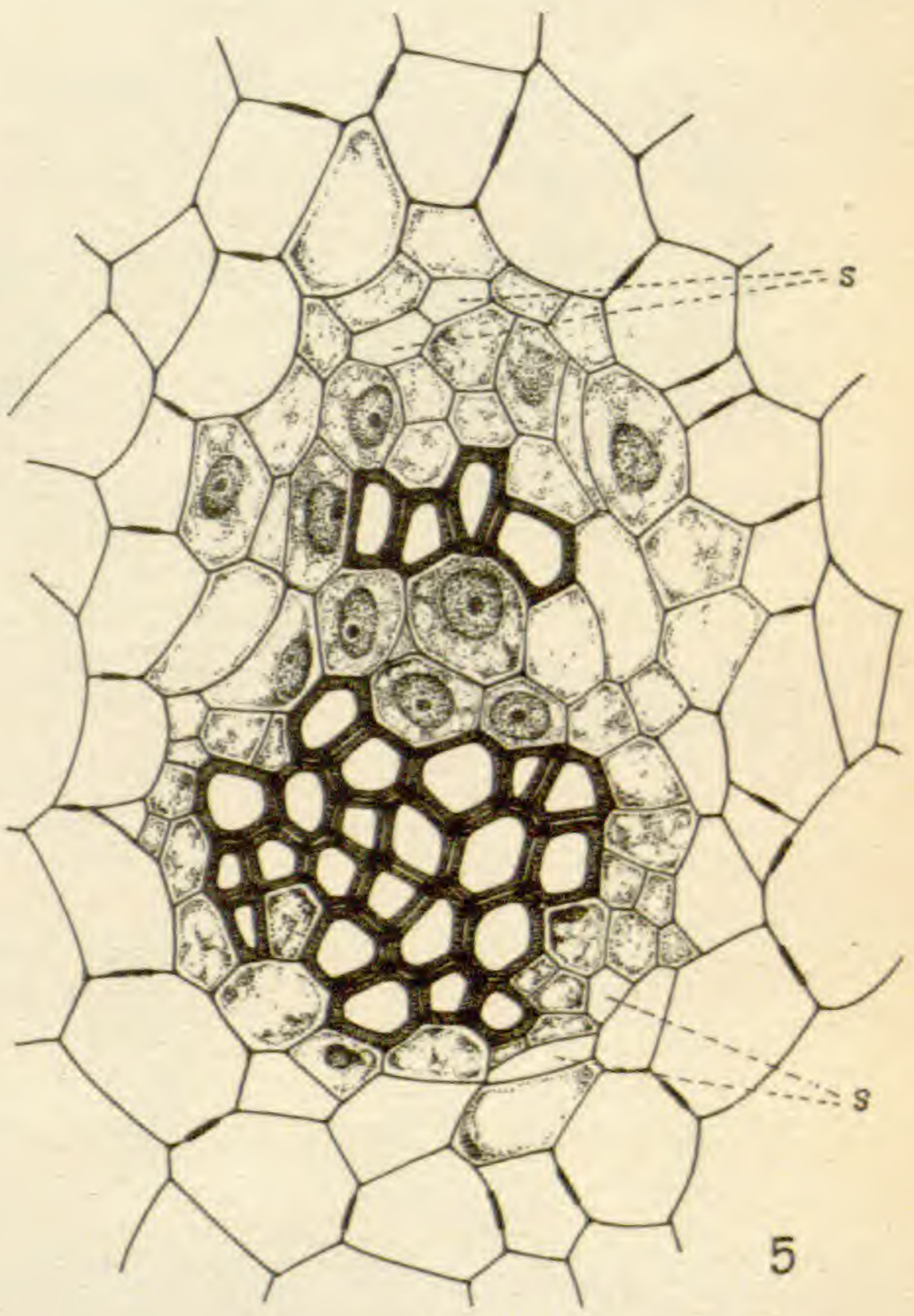

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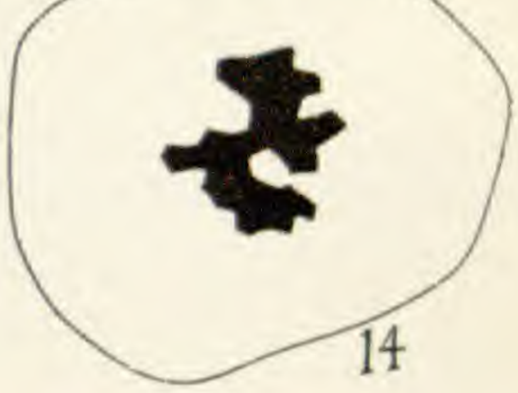
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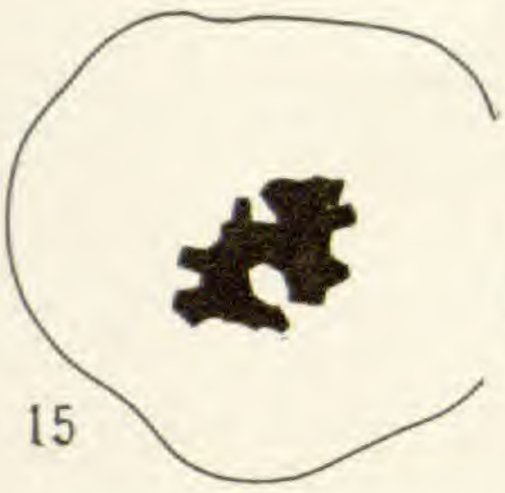
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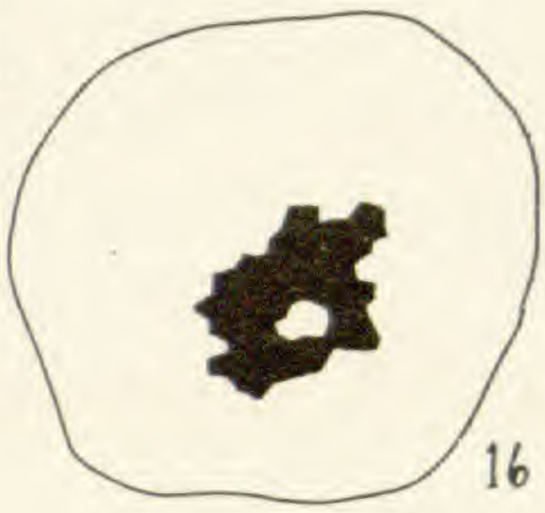
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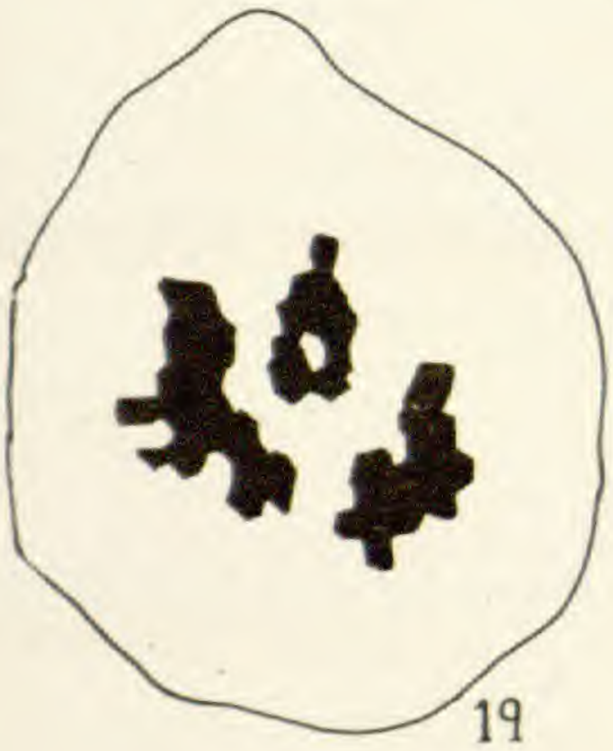
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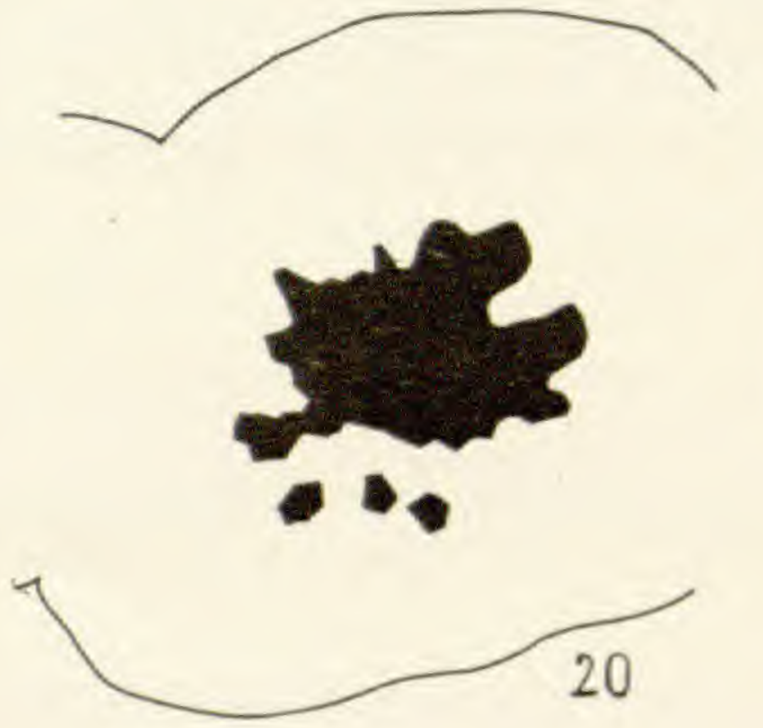
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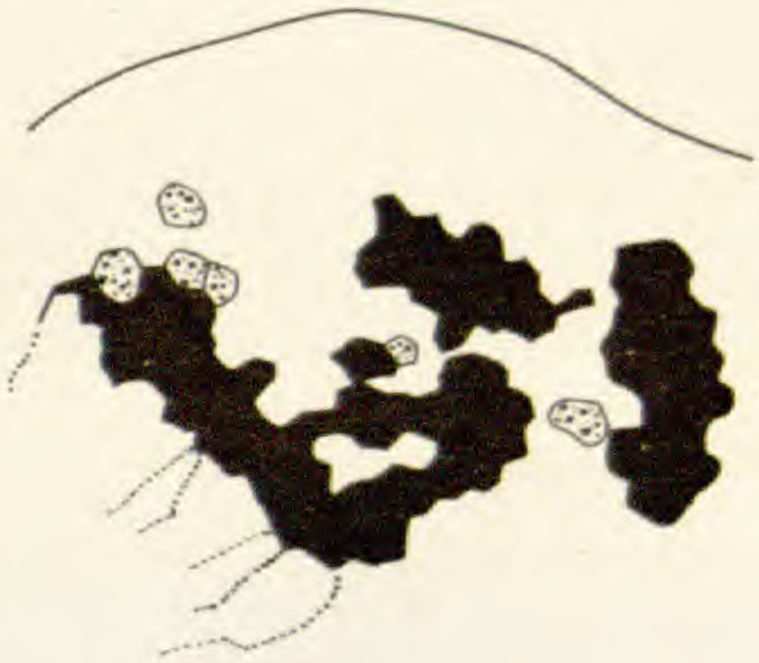
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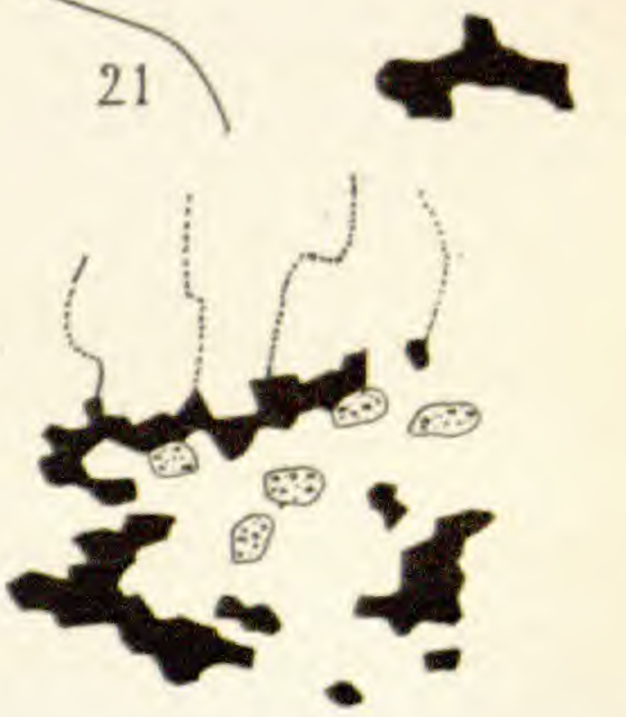
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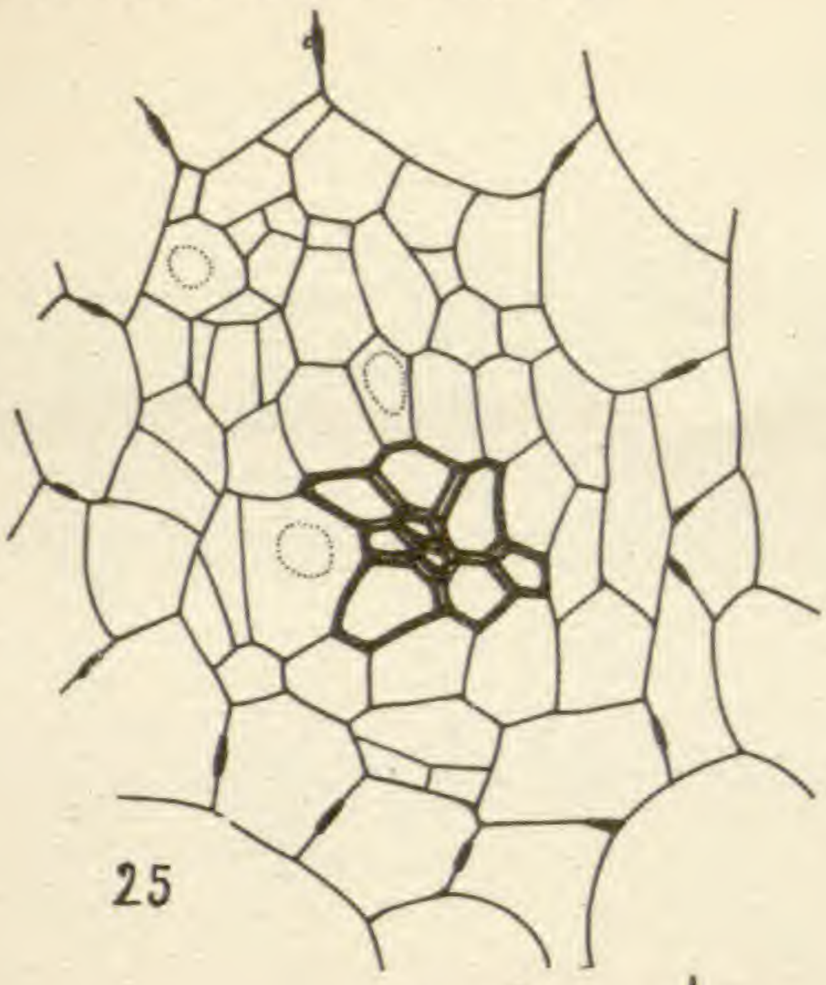

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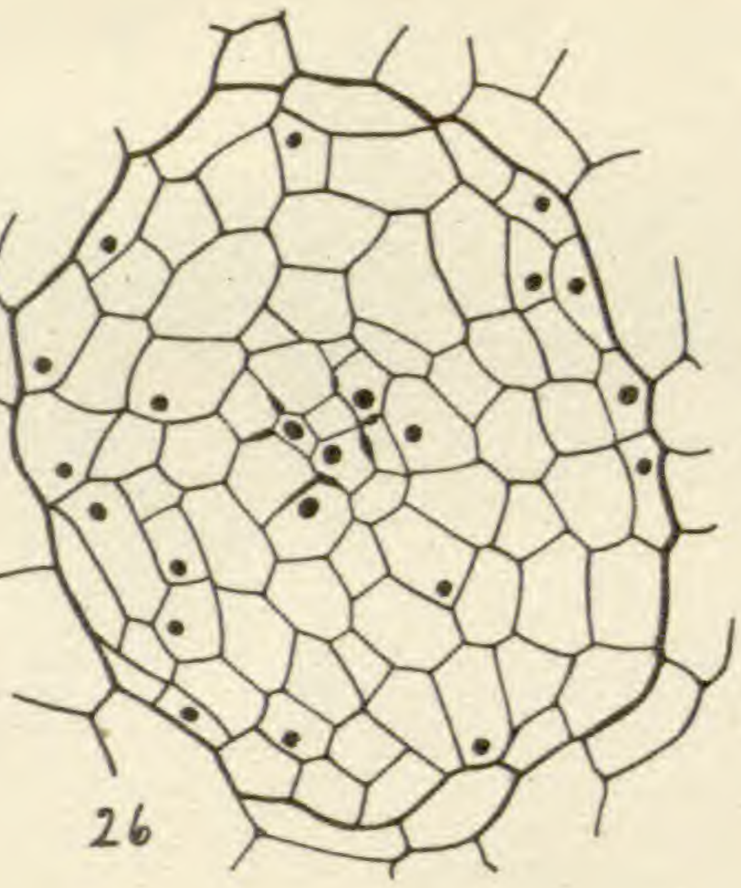

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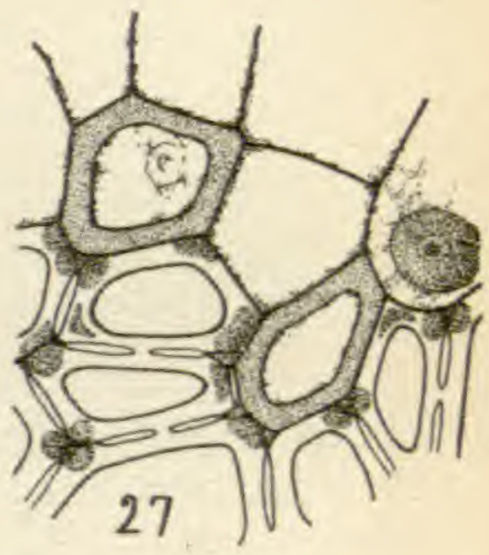

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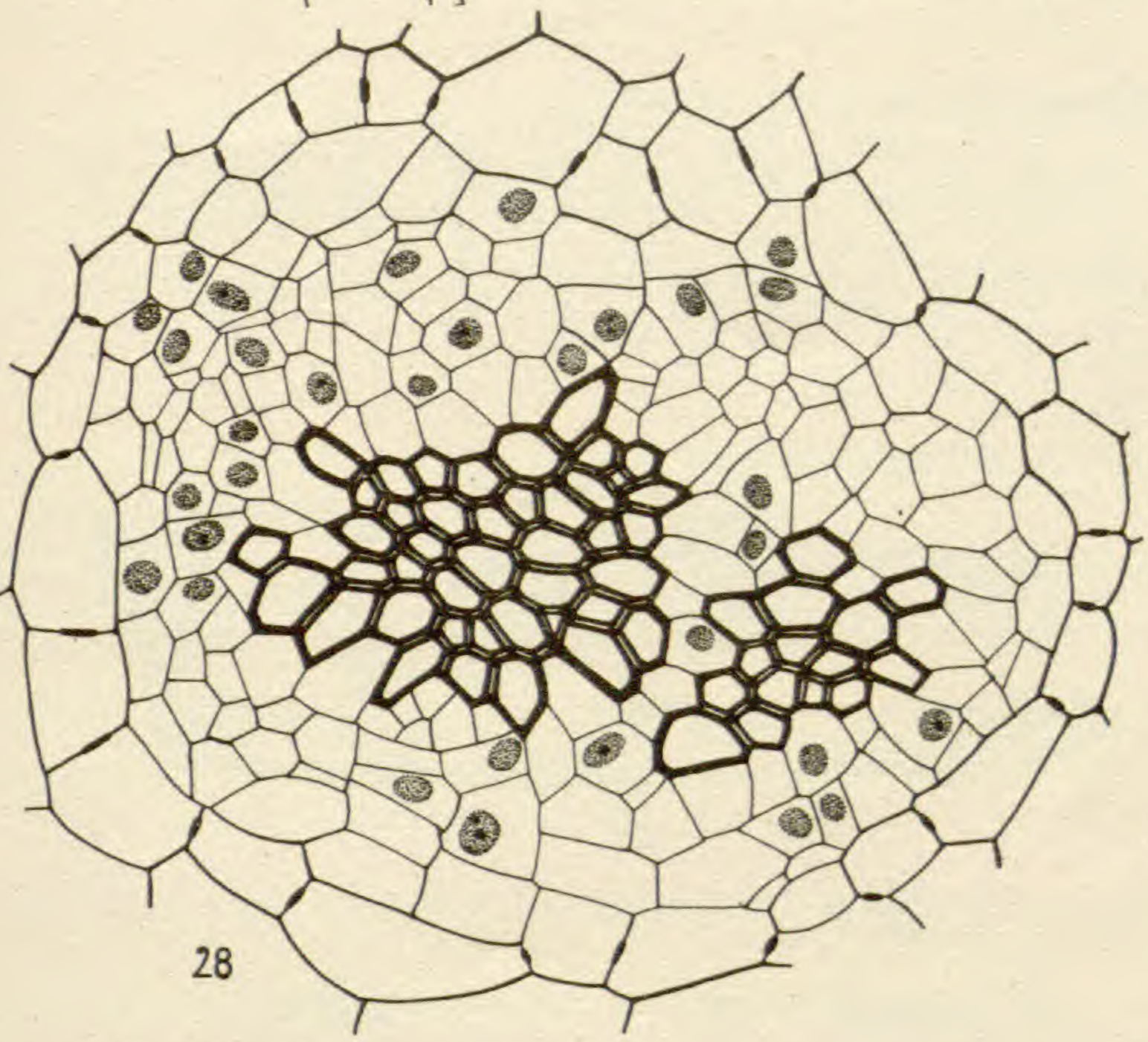

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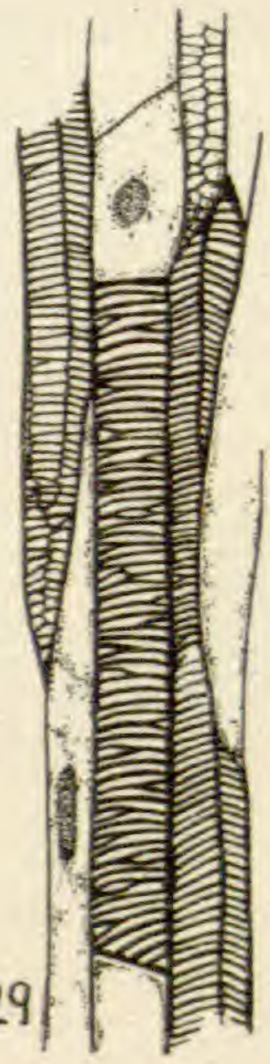

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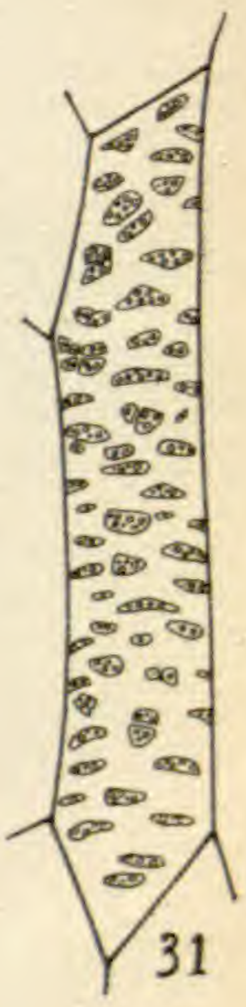

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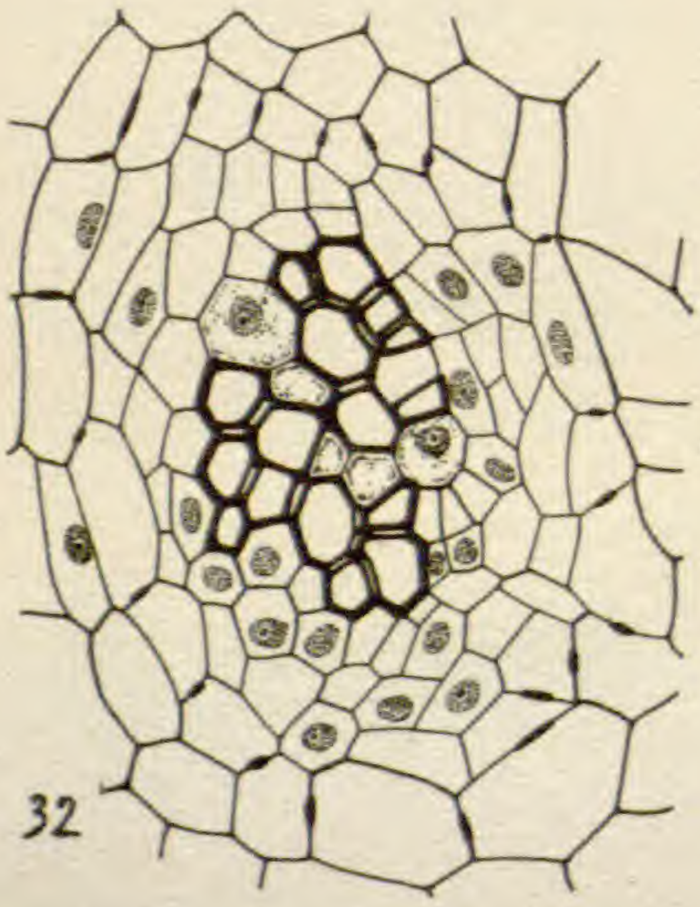

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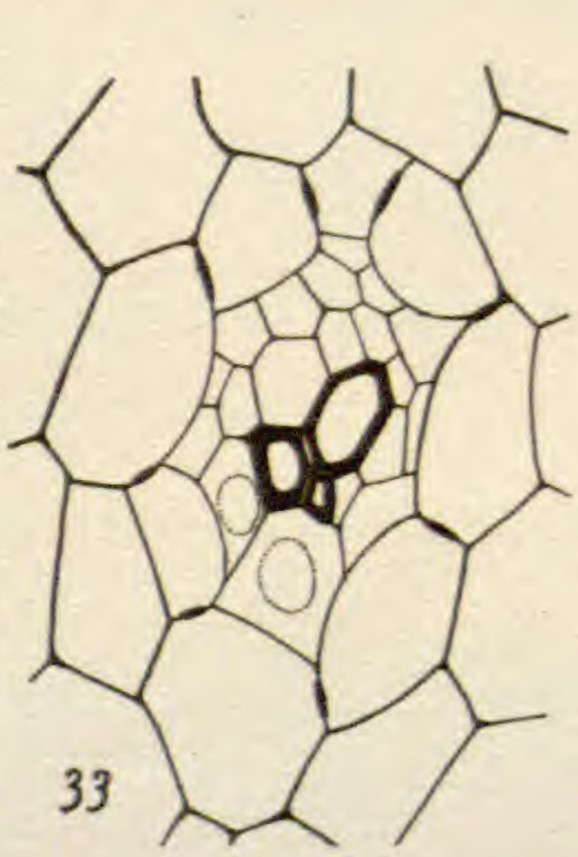

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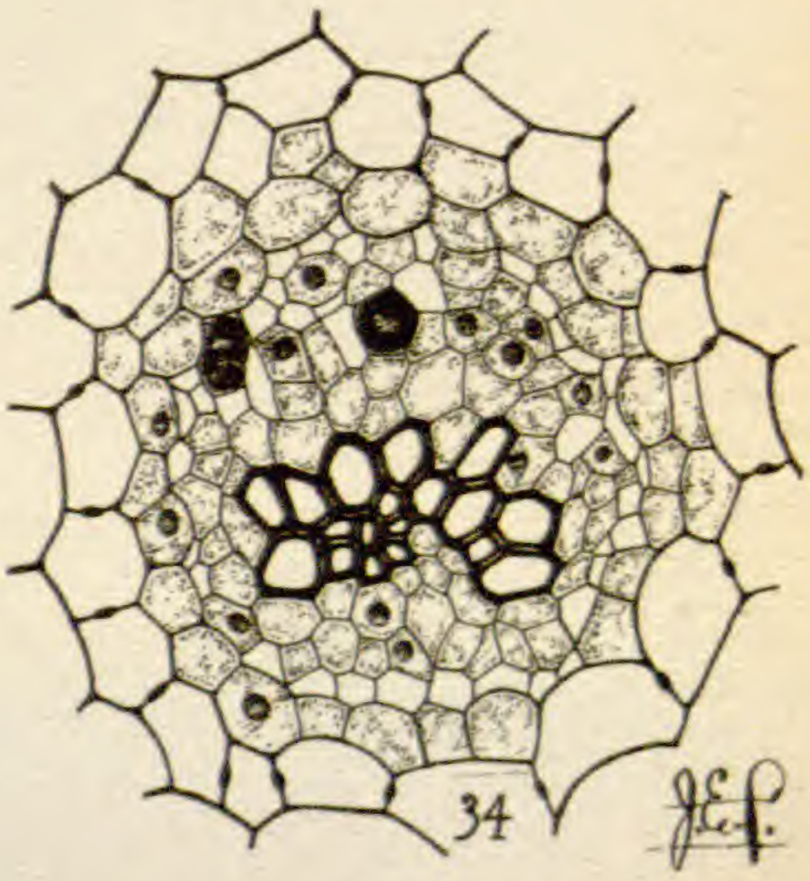

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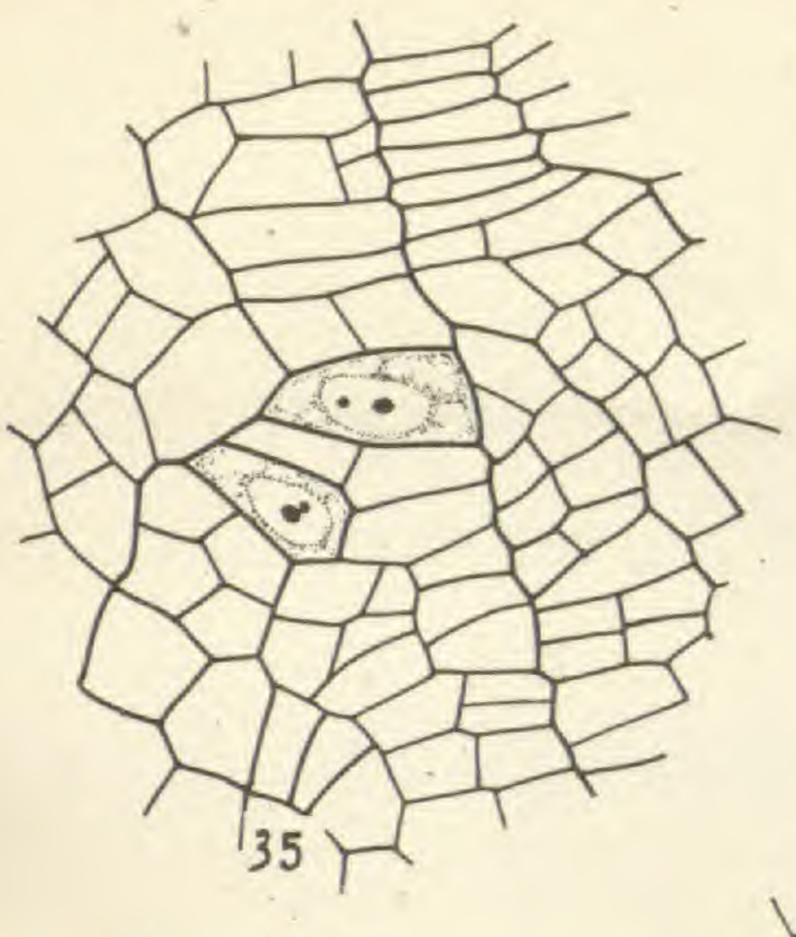
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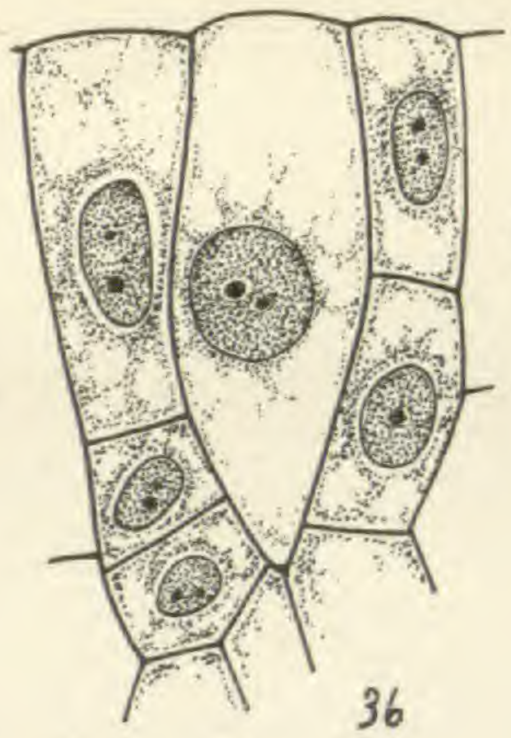
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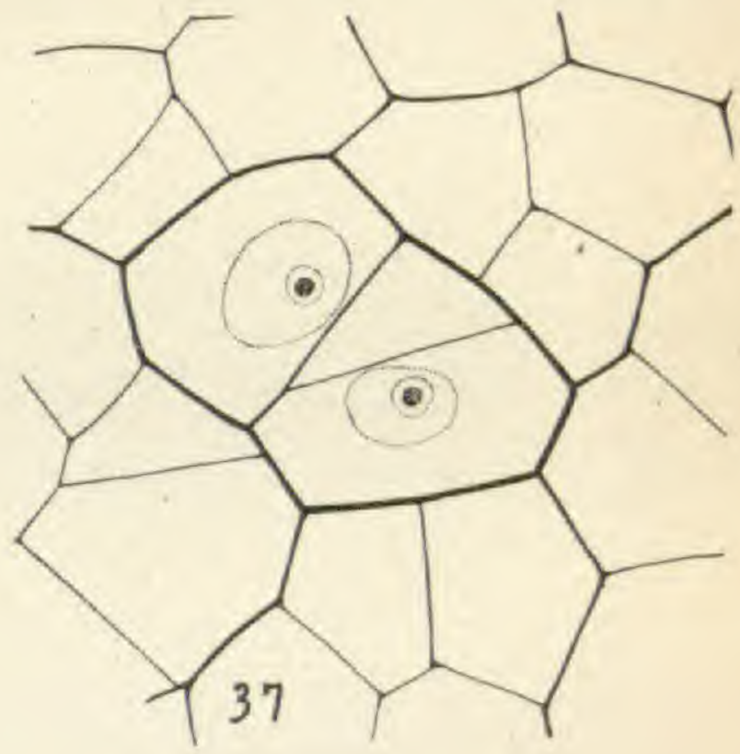
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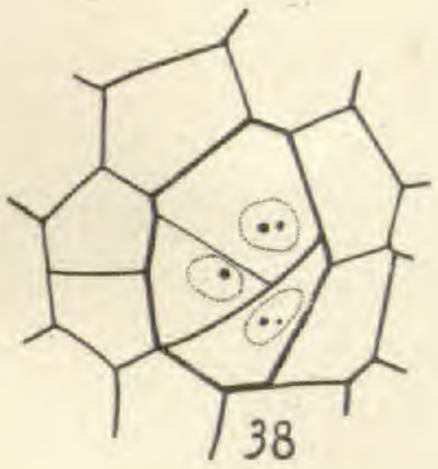
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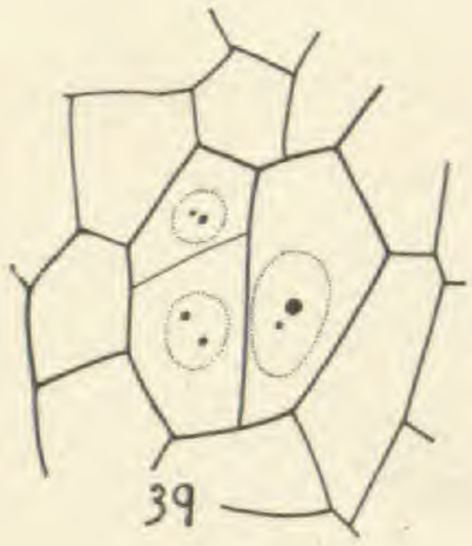
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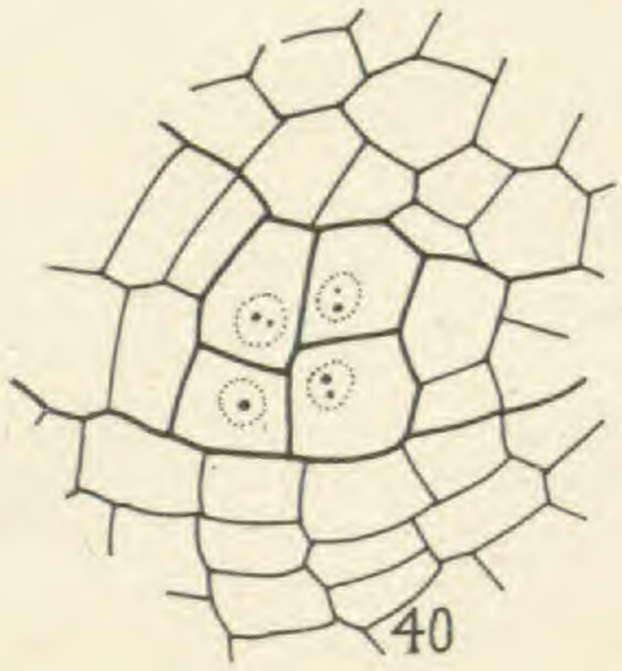
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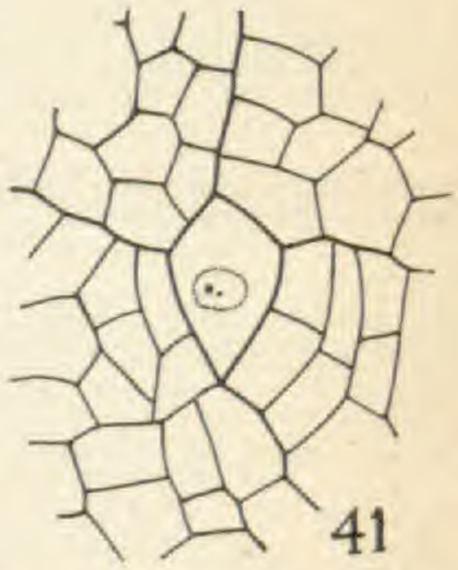
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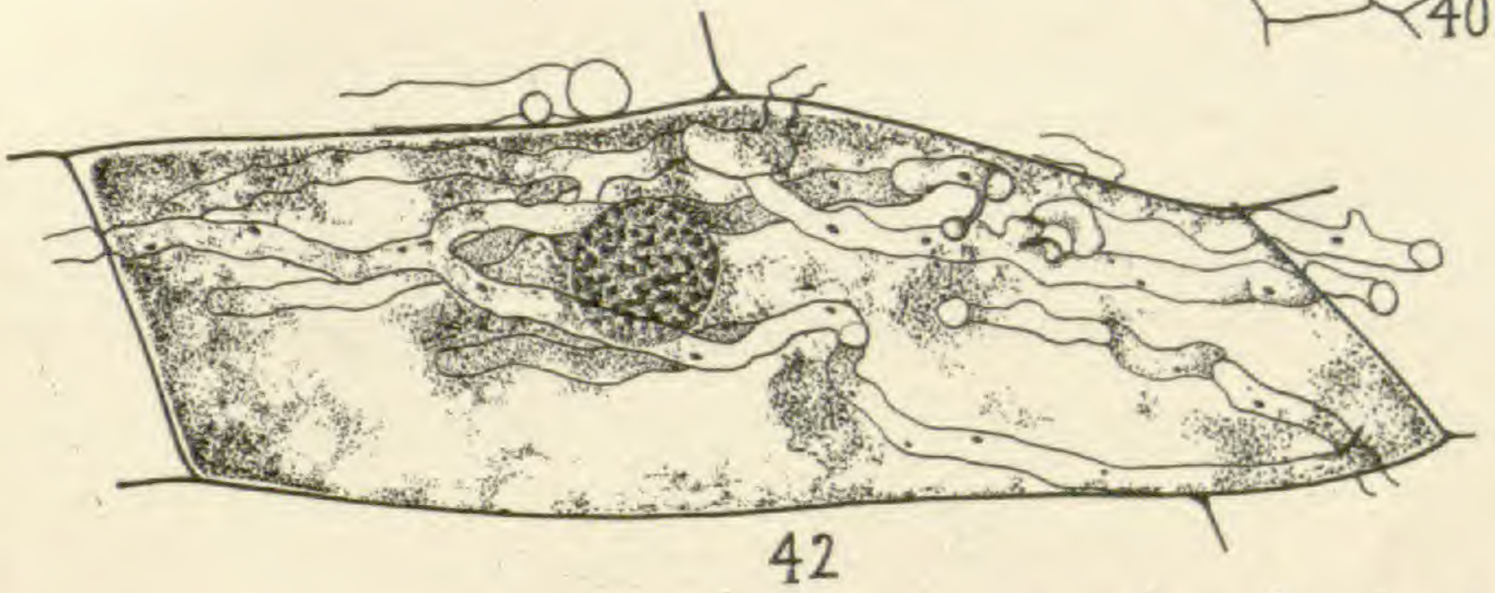
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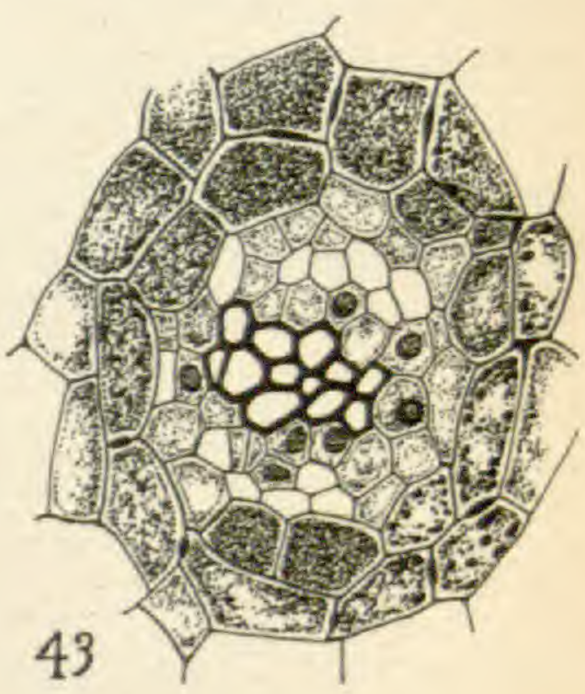
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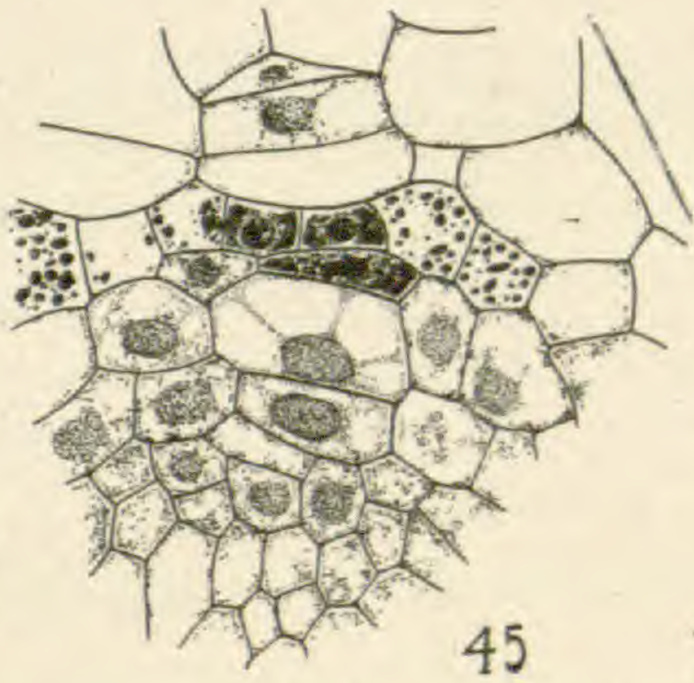
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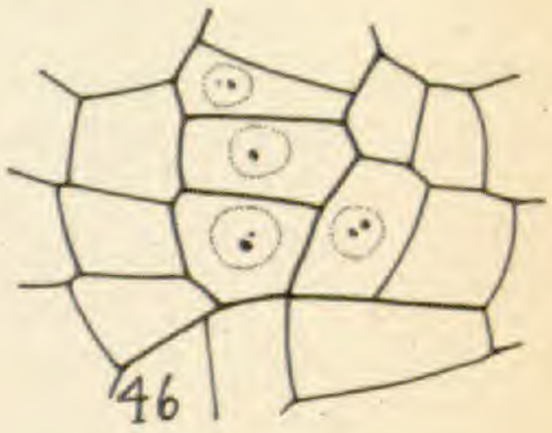
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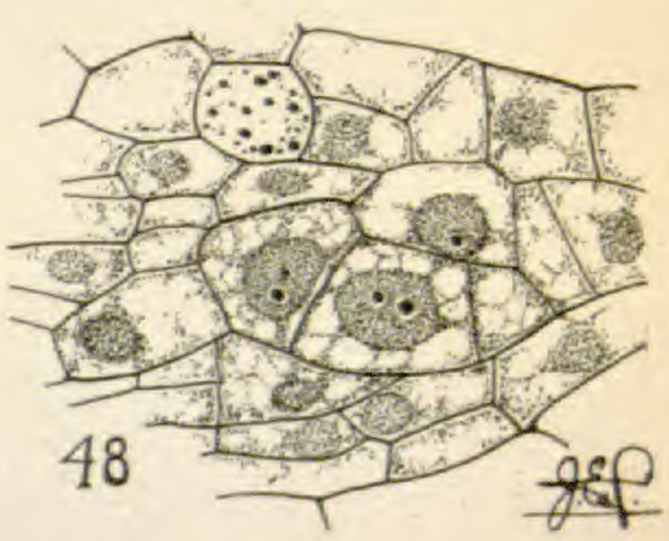
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SUBCORTICAL FORMATION AND ABNORMAL DEVELOPMENT OF STOMATA IN ETIOLATED SHOOTS OF OPUNTIA BLAKEANA

J. G. Brown

(WITH PLATES XXVII–XXX AND ONE FIGURE)

Introduction

Although experiments in the etiolation of plants date back to the activities of Charles Bonnet, their contributions to morphological botany have largely been incidental to the prosecution of physiological studies, and therefore extensive only as regards the number of species subjected to investigation. Even up to the present century, literature contains little more than observations on gross structural changes induced or accompanying etiolation, such as the elongation of internodes and peduncles, the dwarfing of leaves, and the underdevelopment of aerating and conducting tissues. To this literature it is believed that the material discussed in this paper adds several new and important facts. Since other investigators (4) have given reviews of the literature on etiolation, the writer will pass directly to his own studies.

Material

Opuntia Blakeana is a common, low-spreading, prickly-pear cactus on the mesas of southern Arizona, having joints 10 cm. in length, 9 cm. in breadth, and 1–2 cm. in thickness (fig. 1). The joints exhibit purplish areoles. Each areole near the margin and in the middle region of a joint bears one or two brown spines 1–3 cm. long. On the basal portion of a joint and on the lowest joints of the plant, areoles are often without spines. In addition to the spines, areoles frequently bear glochids about half the length of the spines, which are especially numerous near the apex of the joints. Both spines and glochids are of the usual barbed type found in cacti. Many bristles about 4 mm. in length, consisting of a single row of cells, are inserted near the bases of the

spines. Small awl-shaped leaves about 15 cm. in length are also borne just below the spines in the spring, but are soon abscissed. The plant grows in the most exposed situations. It was first described as a new species by ROSE (5) in 1909, and the plant from which the type specimen was obtained furnished most of the

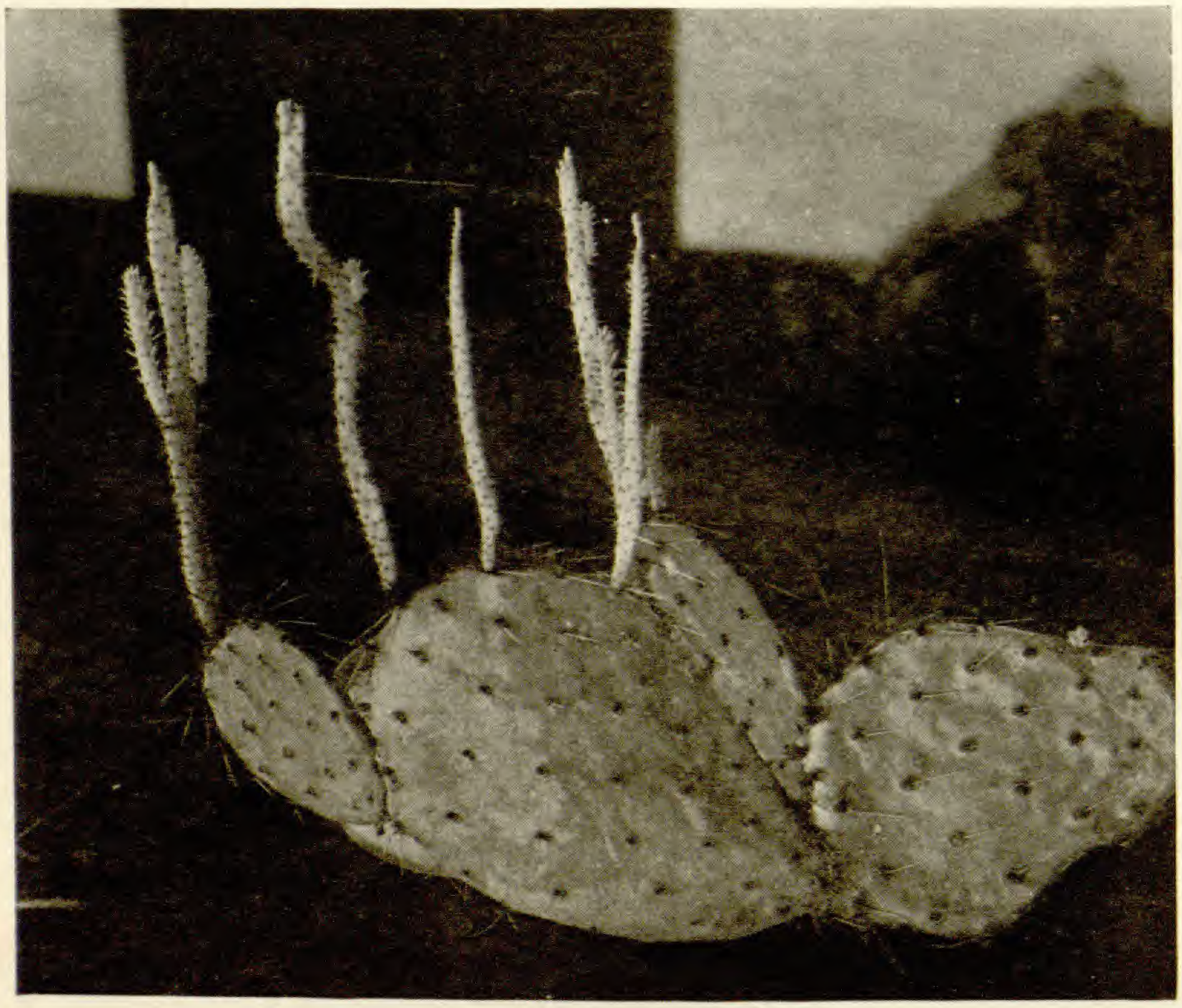

FIG. 1.—Etiolated shoots of *Opuntia Blakeana* sprouting from normal shoots which had been removed from open environment to dark chamber.—Photograph by D. T. MACDOUGAL.

normal shoots, and indirectly all of the etiolated shoots that were used in this study. The latter were grown from joints that had been removed to the dark constant temperature chamber of the Desert Laboratory.

Method

The chamber in which the etiolated shoots were grown has been fully described in publications of the Desert Laboratory,

but for convenience its main features will be given here. It is a small room situated under the floor of the main laboratory, from which light is absolutely excluded by means of an antechamber and two doors, the second door being a trapdoor in the ceiling of the dark chamber. The temperature remained between 56 and 75° F., with a maximum weekly fluctuation of 2° F. The humidity was 80–90 per cent. The room was absolutely dark at all times excepting for the candlelight used while collecting material.

Some of the etiolated shoots were killed in the dark chamber, others were removed to the light and allowed to grow for varying lengths of time before they were killed. The etiolated shoots removed to the light are referred to as etiolated-greened shoots. The killing agents used were chromo-acetic acid and Bensley's mercuric-formalin solution (1), and the material, both normal and etiolated, was then prepared for sectioning in the usual way. LAND'S (3) fixative proved useful in handling large sections. Several stains were used, including safranin-haematoxylon, orange G, and Haidenhain's iron-alum. Because of the mucilaginous nature of cactus material, it was found advantageous to continue the washing operation after the killing agent somewhat longer than is usually necessary with ordinary plant tissue.

Normal shoot

The gross structure of the normal shoot has already been given under the general description of the plant. Cross-sections (fig. 1) showed thick integumentary and palisade layers, an extensive spongy inner cortex, and a stele of elongated bundles. External to the phloem there appeared a mucilage mass which, with the bundle, presented a nail-shaped outline. The integumentary region consisted of cuticle, epidermis, and hypoderm (fig. 11). The cuticle averaged about 11 μ in thickness. Epidermal cells had straight anticlinal walls (fig. 4), and the longest cell measured was about 45 μ. One of the peculiarities of *Opuntia* and a few other cacti observed and mentioned by SCHLEIDEN (6) is the patchy decortication, in which the epidermal cells become active in spots, divide periclinally, and the inner of the two layers of cells thus formed continues to divide until a layer of tissue several

cells thick is present, whose outermost wall is the former external wall of the old epidermis. The tissue developed in this way is eventually entirely cut off, and a new outer epidermal wall is cutinized, so that the new epidermis resembles that which has just been lost.

The stomata were of the regular dicotyl type (fig. 4) and numbered 32–36 per sq. mm. of surface. Because of the thick cuticle they were sunken below the surface of the shoot (fig. 14). Below the epidermis extended the hypoderm, about six cells in thickness (figs. 11, 14). The first layer of the hypoderm consisted of a sheet one cell thick, with a large crystal of calcium oxalate in almost every cell. The remainder of the hypoderm was made up of about five layers of stone-cork cells. In places the crystal-containing cells almost closed the air chambers just under the stomata. The stone-cork cells were deeply pitted. They gave place abruptly to the palisade tissue with its long tubelike columns of cells, which was followed by the spongy cortical region reaching to the stele. The most external chloroplasts occurred in the palisade cells, where they were numerous on the lateral and end walls. Large air chambers extended from the stomata inward through the hypoderm into the palisade tissue, and the intercellular spaces were extensive in both palisade and spongy inner cortex. The structure of the stele will be described in a future paper.

Etiolated shoot

The appearance of the etiolated shoots presented a marked contrast with that of the normal ones (fig. 1). They were pinkish at first, but later changed to a very light green. They were longer than the normal shoots and more or less flattened in cross-section (fig. 3). Numerous sessile leaves like those of the normal plant in form were produced, which persisted for a short time. The longest measured was 7.5 mm. Spines, bristles, and glochids were grouped in a normal manner but were reduced in size, the spines averaging 3 mm. in length, the bristles about 1.5 mm., and the glochids intermediate between the two.

Unlike that of the normal plant, the epidermis of the etiolated shoot was without cuticle (fig. 13), although the walls showed

more or less cutinization. Individual cells varied in form and size from the base to the apex of the shoot. Those in the apical region were much elongated (fig. 6), some of them measuring 175 μ. Their lateral walls were straight, and their shorter end walls were wavy in outline. In the basal region the epidermal cells were not longer than 85 μ, and nearly all of the walls were slightly wavy when seen from the surface of the shoot (fig. 5).

However striking the changes in external form and gross structure due to etiolation may appear, the internal changes were even more interesting. When the surface of the etiolated shoot was examined under low magnification, numerous small elevations were observed which reflected the light. Closer investigation showed these structures to be minute papillae, each one bearing a stoma at the apex (figs. 13, 25, 27). Their nature was better seen in longitudinal and transverse sections of the shoot (figs. 25, 26), and they were usually found to be epidermal, the entire structure arising in most cases from a single cell; in others, chiefly from a single cell, but augmented to some extent by division of neighboring cells of the cortex. The papillary initial appeared to be analogous to a stoma mother cell. Evidently the stimulus to division had continued to act on the stoma mother cell and its progeny for some time and had met with a response, even after cutinization of the surface cell walls had occurred, for in one instance the resulting structure, unable to grow outward, had pushed inward among the undifferentiated cells of the cortex (fig. 28). The first division of the papillary initial was either a vertical one, as in the ordinary process of stoma formation (figs. 7–9), or else the cell became papillate and divided by an oblique wall (figs. 18, 19). The lower of the two cells thus formed then divided by a vertical wall, and the upper cell followed with a transverse division (fig. 20). After this the walls were chiefly oblique. Eventually two guard cells were cut off, which, seen from the surface, resembled the guard cells of a normal stoma (fig. 27), but which were more or less wedge-shaped in transverse sections of the shoot (figs. 21–25). The mature papilla might be compared to a hydathode in surface view, but in internal structure and in origin it was very different; the tissue was not glandular, no

vascular or conductive elements of any kind ended near it, and it usually originated from a single epidermal cell. Although large and solid papillate structures were most numerous, every transition was found between that type and the simplest form of stoma. Thus some papillae consisted of only a few cells in addition to the stomatal guard cells (fig. 26), and there was every gradation in size from this up to a papilla of dozens of cells. Some of the papillae were hollow also, and it appeared that the cavity had formed by the breaking down of the internal cells into a mass resembling mucilage. The simplest stomata consisted of guard cells with no differentiated auxiliary cells (figs. 32, 33), and often with neither intercellular space nor air chamber below. A few normal stomata had mucilage masses just underneath the stomatal opening.

Another peculiar situation was suggested by the occasional occurrence of a perfectly developed stoma lying almost under the margin of decorticating patches, and it was finally disclosed after a careful search. Three stomata were found developing under several layers of cork cells, one of which had guard cells just beginning to split apart (fig. 30). So far as can be discovered, the subcortical formation of stomata has never been reported. Perhaps it does not occur outside of the cacti.

In numbers the stomata of the etiolated shoot ran far below the normal plant, 12–16 being the maximal numbers found per sq. mm., even in the lower part of the shoot where they would be expected to be most numerous, in agreement with the nature of the epidermis which here most closely resembled that of the normal shoot. The stomata were mostly open.

The regions of a cross-section of the etiolated shoot also contrasted sharply with those of the normal stem (figs. 1, 3, 11, 13, 17). That the cuticle was absent has already been mentioned. The epidermis consisted of cells much broader than normal and much better supplied with protoplasm. Large budding chloroplasts were present which resembled chains of yeast cells in form, and varied in size from 30 μ to granules too small to be studied with the highest available power of the microscope (fig. 17). Below the epidermis there was neither a crystal-containing layer

nor stone-cork hypoderm, nor was a palisade tissue differentiated, but two general physiological regions could be recognized: an outer leucoplast-containing one 3–5 layers of cells thick, and an inner starch-containing one reaching to the stele (fig. 13). The leucoplasts of the outer cortical region were actively budding, like the chloroplasts of the epidermis, but were mostly smaller, and the smallest sizes increased in number in proportion to the distance from the surface of the shoot. Air spaces were much less extensive than in the normal cortex, and were often formed abnormally, as previously described.

Etiolated-greened shoot

Etiolated shoots placed in the laboratory windows and those transferred to the open presented similar changes in structure, but the changes were more rapid in the latter environment. Decortication removed the abnormal stomata with the papillae, and none reappeared (figs. 15, 29). Chloroplasts quickly disappeared from the epidermal cells. The whole shoot presented a shrunken appearance, due not only directly to the water loss from the almost unprotected tissues, but also to the actual death of many of the cells in the outer cortex (figs. 15, 16), a process by which air cavities were quickly enlarged. As cutinization progressed in the epidermis, and the turgidity of the cortical cells gradually became restored, the whole topography of the cross-section changed, for palisade tissue had appeared (fig. 12). Intracellular changes also occurred in the cortical cells. The chloroplasts were reduced in number as compared with the leucoplasts in the outer cortex of the etiolated shoot. They were regularly rounded in form and were present in cells as deep as the stelar region (fig. 12). They were necessarily confined to the peripheral region of the cell because of extensive vacuolization, and were found on end walls and lateral walls.

The new branches that appeared from the buds that had formed on the etiolated shoots before their removal to the light were larger, both in breadth and thickness, than the branches of the etiolated stems; and spines, glochids, and bristles were more like those of the normal plant in size and general appearance.

Discussion

The preceding description of the results obtained in the etiolation of *Opuntia Blakeana* suggest for discussion the factors concerned in the development of cuticle; the outline of epidermal cells; the number, origin, and development of stomata; the formation of palisade tissue; and the appearance of air spaces.

Cuticle formation, it has been suggested, approaches a maximum when transpiration is great in amount, and when it is high in proportion to absorption (2). It has also been stated that cuticle formation is favored by growth in concentrated nutrient media. Both factors were probably operating in these experiments. The etiolated shoots certainly transpired much less than they would have done had they been grown in the open, for they promptly wilted when transplanted to the latter environment. Gradual increase in the osmotic pressure of the sap in the joints from which the etiolated shoots obtained their nutrient supply must have occurred, but it was evidently insufficient to induce the development of cuticle. MacDougal found no cuticle formation in any of the numerous plants with which he experimented.

The changes mentioned in connection with the outline of the epidermal cells have been observed by many investigators. Whether light is a factor in determining the shape of the cell walls in the epidermis could not be determined, for an attempt was not made to reduce transpiration when etiolated shoots were removed to an outdoor environment in order to separate the two factors. Mesophytic conditions in the dark chamber may be considered favorable to crenated walls in view of the results of other workers (2).

Decrease in the number of stomata per unit of surface area is in general harmony with the results of numerous investigators. Six or seven times as many epidermal cells per unit of surface appeared on the normal shoots, while the stomata were two to three times as numerous, compared with the etiolated ones. In his experiments with *Opuntia Opuntia*, MacDougal found that the stomata on the etiolated shoots were reduced in size. Those measured in *Opuntia Blakeana* were not different in size from the stomata of the normal plant, excepting a few freakish forms of stomata.

Development of stomata under cortical tissue is a phenomenon that is difficult to explain. COWLES states that although the factors inducing the appearance of stomata are unknown, evidence is not lacking that light favors their development, and that stomata are abundant where transpiration is vigorous, and absent where it is reduced or wanting. Neither factor could have greatly influenced the development of subcortical stomata in a direct way in this case, for light was absolutely excluded and transpiration was much reduced, owing to the increased moisture content of the air in the dark chamber and the covering of cortical cells. In connection with the light factor, however, there is another interesting possibility. In MACDOUGAL's experiments, seedlings of *Aesculus*, whose basal internodes were briefly illuminated, developed laminar bodies in internodes formed some weeks after the stimulus had been given, which were entirely lacking in the absolutely etiolated seedlings. He states that "the stimulative effect of illumination . . . . may be received by one portion of the body and transmitted to another, and the impulses may even be communicated to organs not actually formed at the time the stimulating rays were received." The last resort appears to be to ascribe the phenomenon to some internal factor such as is included under the term heredity.

The papillary structures appear to be peculiar to the etiolated shoots of *Opuntia Blakeana*. That they result from the division of a cell analogous to a stoma initial seems to be a logical conclusion, since all stages are found, from the normally developed stoma with two guard cells and two adjacent cells originating from one initial, to the elevated structure consisting of many cells around and below the guard cells, all developed from one initial. The fact that the first division may be a periclinal one appears to be a matter of detail that does not exclude such an interpretation. Some stimulus, possibly previous illumination or some internal stimulus, starts the division process, which is favored by the increased moisture of the dark chamber and by an abundant food supply from the normal shoots at the base of the etiolated ones. Once started, the divisions continue until the cutinization of the epidermal walls offers sufficient resistance to check the growth

of the papilla. The appearance of the abnormal structure shown in fig. 28 seems to favor such a conclusion.

Among the theories advanced to explain palisade development are the light theory, the transpiration theory, and the lateral pressure theory (2). No attempt was made to separate the light and transpiration factors when the etiolated shoots were transplanted. Both factors were increased upon transferring the etiolated shoots to the light. Lateral pressure must have been reduced, for the cortical tissues had a minimum of intercellular air spaces in the dark chamber and a maximum in the etiolated-greened condition. Furthermore, the collapse and death of many cortical cells after exposure of the shoots to an outdoor environment must have further reduced lateral pressure. Another factor may have been operative. The partially cutinized epidermal walls resisted collapse, judging by the appearance of sections of fresh and killed tissues, and this cylinder of epidermis, as it gradually decreased its water loss by increased cutin secretion, must have set up an outwardly directed strain that could not fail to influence the shape of the cells attached to it.

The appearance of large intercellular spaces in the outer cortex is closely related to palisade development which has already been briefly discussed. Shrinking and subsequent turgidity and the death of cortical cells were important factors in increasing the intercellular spaces and enlarging air chambers. All of these factors are probably active in various plants growing in a natural environment in the southwest. It has already been shown for succulents that shrinkage and expansion are marked with the change from wet to dry seasons (7). An investigation of plants that survive continued dry winds would probably reveal intercellular changes in the cortex similar to those observed in these experiments in the etiolated-greened shoots.

Summary

1. This paper deals with a comparison of the appearance, form, and structure of normal, etiolated, and etiolated-greened shoots of *Opuntia Blakeana*, a prickly-pear cactus of the southwest.

2. The flat spiny joints of the normal plant, when carried into the dark chamber, produced roundish, light green, elongated, etiolated shoots which differed remarkably in form and structure from those of the normal plant, and which exhibited structural changes when transplanted in an outdoor environment that brought them to resemble the normal shoot.

3. The etiolated shoots lacked a cuticle, developed papillate structures and stomata abnormal in form and position, and lacked the cortical differentiation so characteristic of the normal shoot.

4. The etiolated-greened shoots lost water rapidly at first. Their air spaces increased rapidly by active intercellular splitting of walls and by the collapse and death of cells; then a cuticle appeared; cortical cells elongated, forming palisade tissue; and in other respects the shoots approached the normal ones in structure.

The study presented in part in this paper was made possible through the interest of Dr. D. T. MacDougal, Director of the Desert Laboratory, who furnished the etiolated material used, and whose friendly criticism constituted a continual source of inspiration. Acknowledgments are also due Professors John M. Coulter, Charles J. Chamberlain, and W. J. G. Land, of the University of Chicago, for numerous helpful suggestions.

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EXPLANATION OF PLATES XXVII–XXX

PLATE XXVII

FIG. 1.—Cross-section of normal shoot showing topography: *a*, integumentary region; *b*, palisade region; *c*, mucilage mass at peripheral side of vascular bundle; *d*, stelar region; ×0.5.

FIG. 2.—Cross-section of etiolated-greened shoot showing topography: *a*, epidermis; *b*, cortex; *c*, leaf trace; *d*, vascular bundle; *e*, stele; ×12.

FIG. 3.—Cross-section of etiolated shoot showing topography; same ettering as fig. 2; ×12.

FIG. 4.—Epidermis from normal shoot after removal of cuticle; ×395.

FIG. 5.—Epidermis from basal region of etiolated shoot; ×395.

FIG. 6.—Epidermis from apical region of etiolated shoot; papillae not yet fully developed; ×395.

FIGS. 7–10.—Normal development of stoma in epidermis of etiolated shoot; fig. 8 is vertical section of stage shown in fig. 7; ×525.

PLATE XXVIII

FIG. 11.—Part of transverse section through outer region of normal shoot: *a*, epidermis with layer of cuticle above; *b*, hypoderm; *c*, palisade region; first layer of hypoderm is a crystal-containing sheet; 2 air chambers shown; ×85.

FIG. 12.—Part of transverse section through etiolated-greened shoot reaching from epidermis into stele; palisade tissue has appeared; round bodies represent chloroplasts; *a* and *c* as in fig. 11; *d*, inner spongy cortex; ×85.

FIG. 13.—Part of transverse section of etiolated shoot reaching from epidermis into stele; papilla shown in upper right-hand corner and part of mucilage mass in lower left-hand corner; black dots represent leucoplasts, and circles represent starch grains; ×85.

FIG. 14.—Detail of integumentary region of normal shoot; lettering as in fig. 11; ×525.

FIG. 15.—Part of transverse section of etiolated shoot shortly after removal to light; decortication of outer layers of shoot involving a stoma in progress; *a*, future surface wall of new epidermis; ×525.

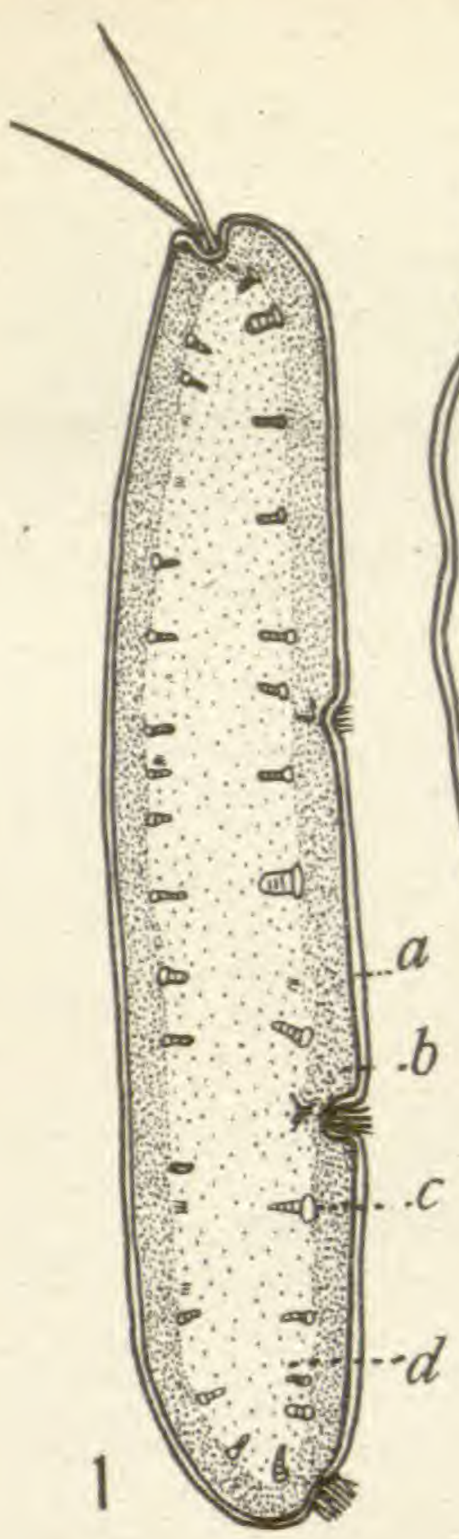
FIG. 16.—Detail of stage of adjustment of etiolated-greened shoot shown in fig. 12; remains of dead cell showing below, projecting into air space; ×525.

FIG. 17.—Detail of transverse section shown in fig. 13; reticulate bodies are nuclei; irregular bodies are chloroplasts and leucoplasts; large, round, lightly stippled bodies are grains of storage starch; ×525.

PLATE XXIX

FIGS. 18–25.—Development of papilla as seen in transverse sections of etiolated shoot: *a*, inner wall of initial cell; *b*, first transverse wall; *c*, stoma; fig. 19, ×390; figs. 20, 22, ×245; all others, ×525.

FIG. 26.—Vertical section of young papilla cut parallel with long axis of etiolated shoot, showing side view of guard cell; ×525.



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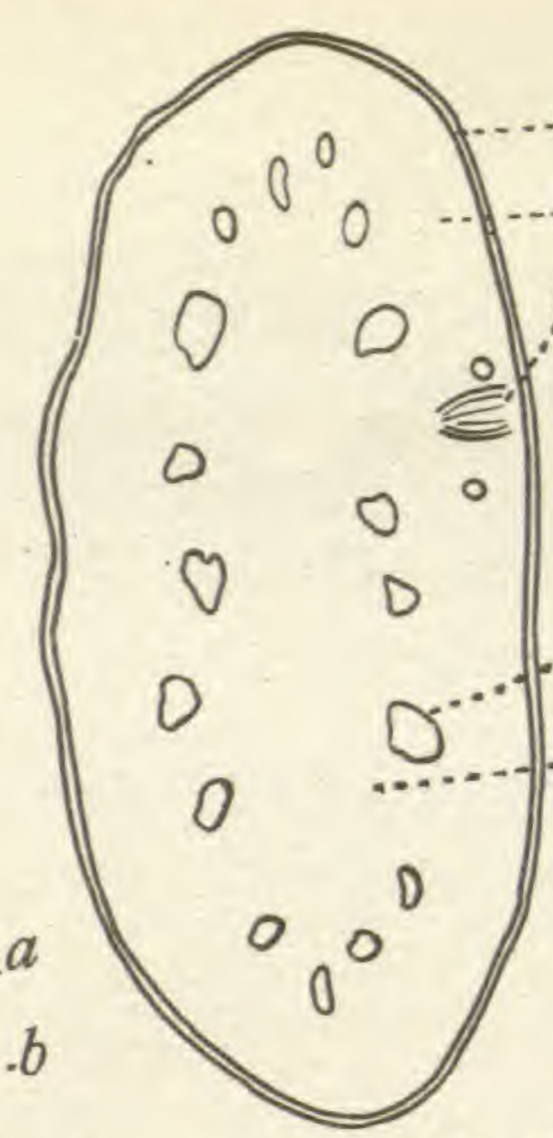

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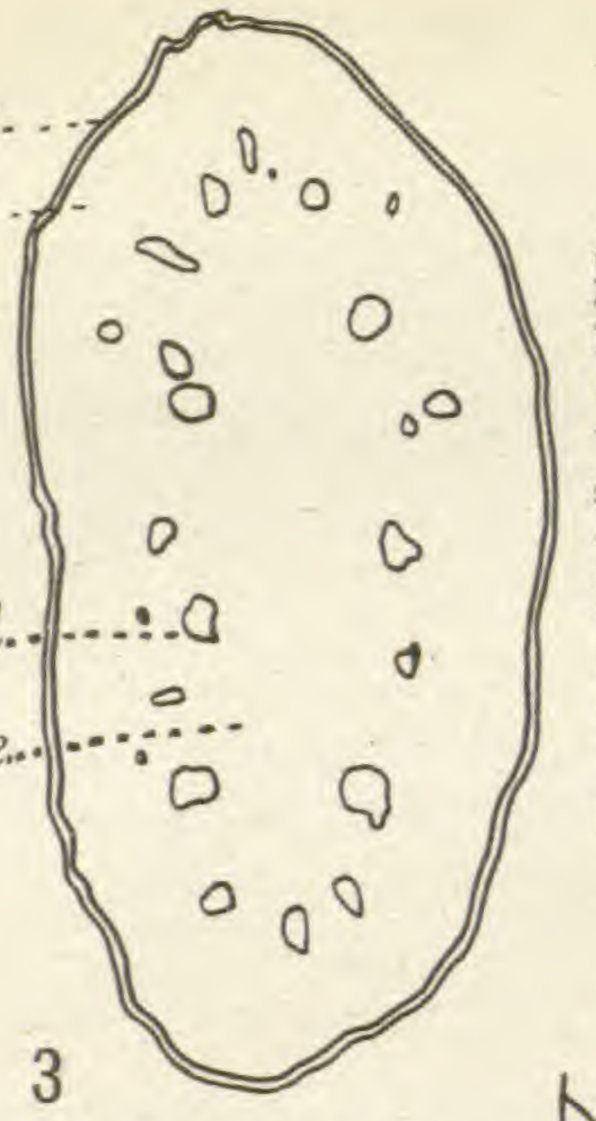

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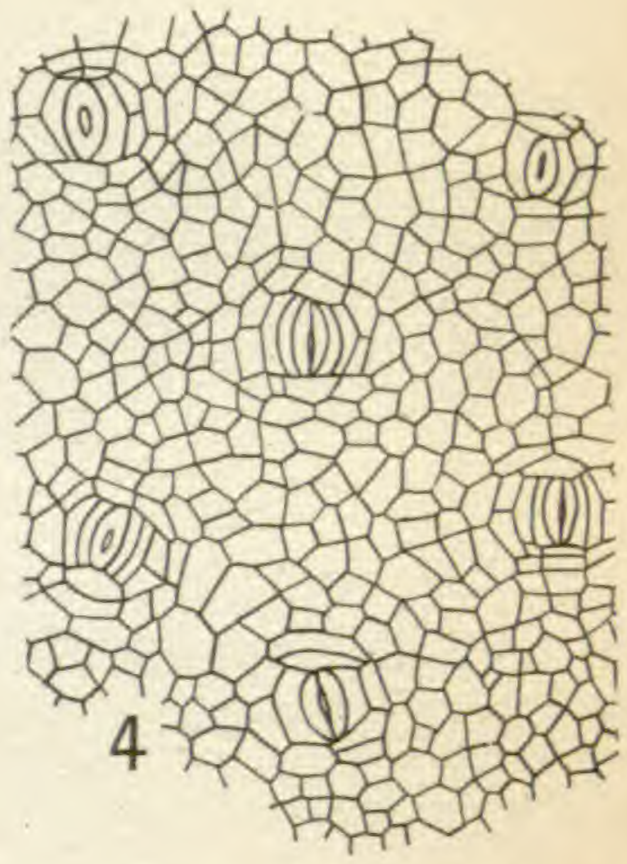

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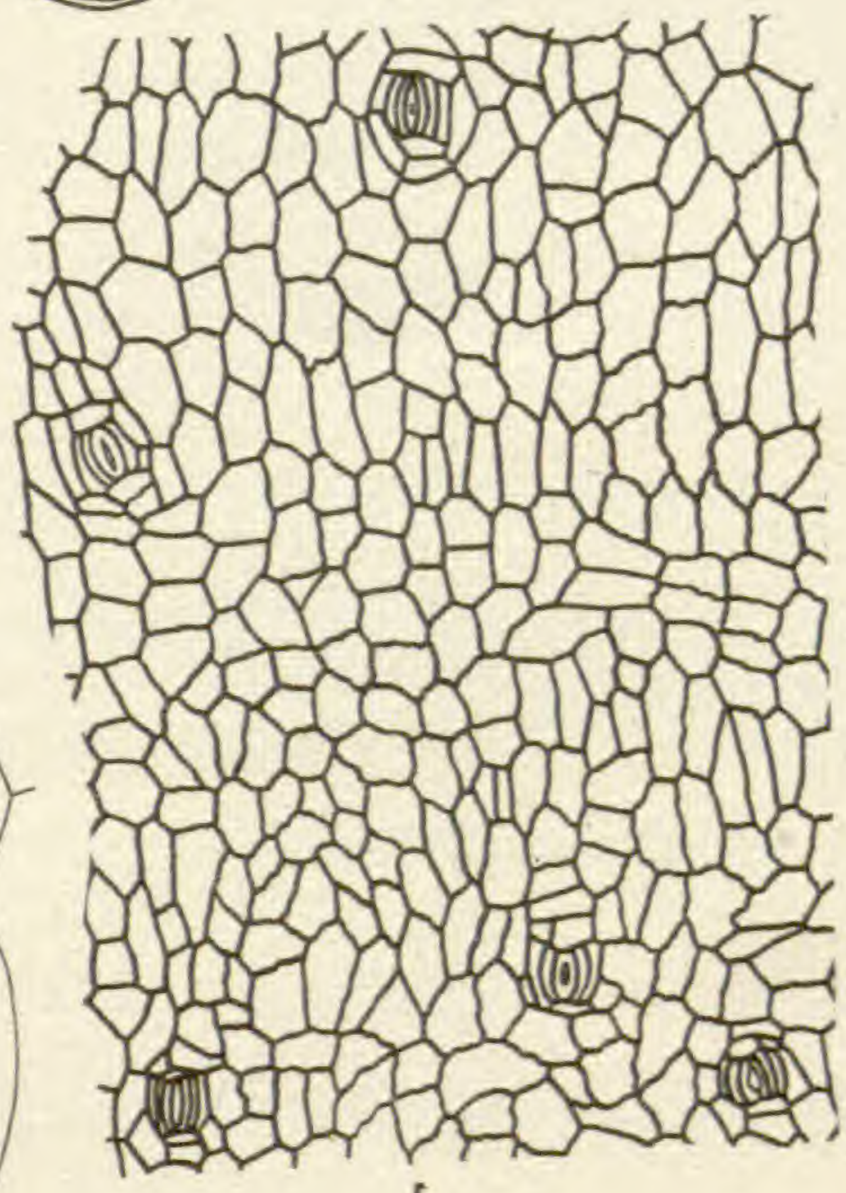

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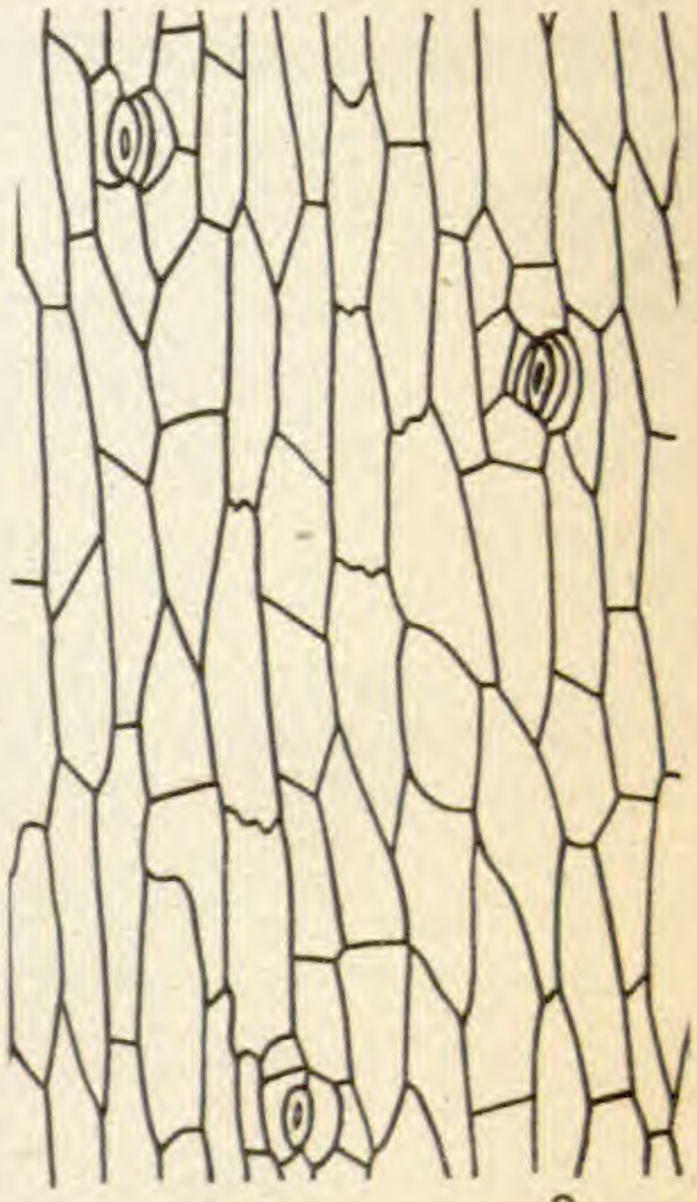

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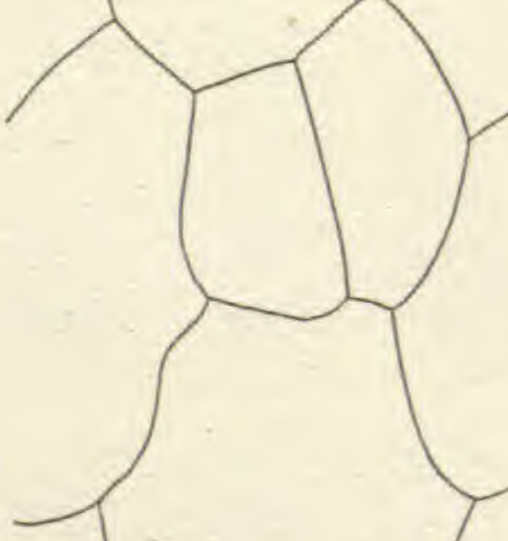

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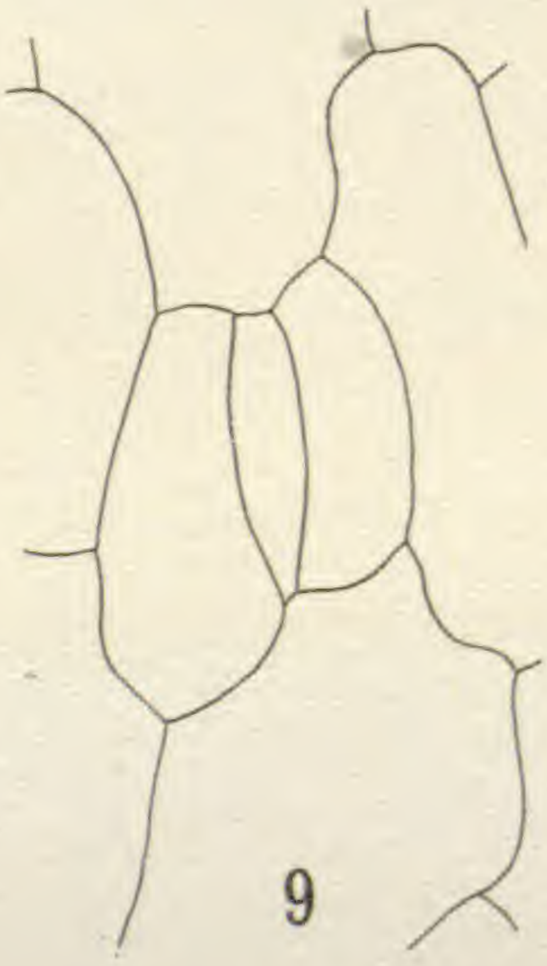

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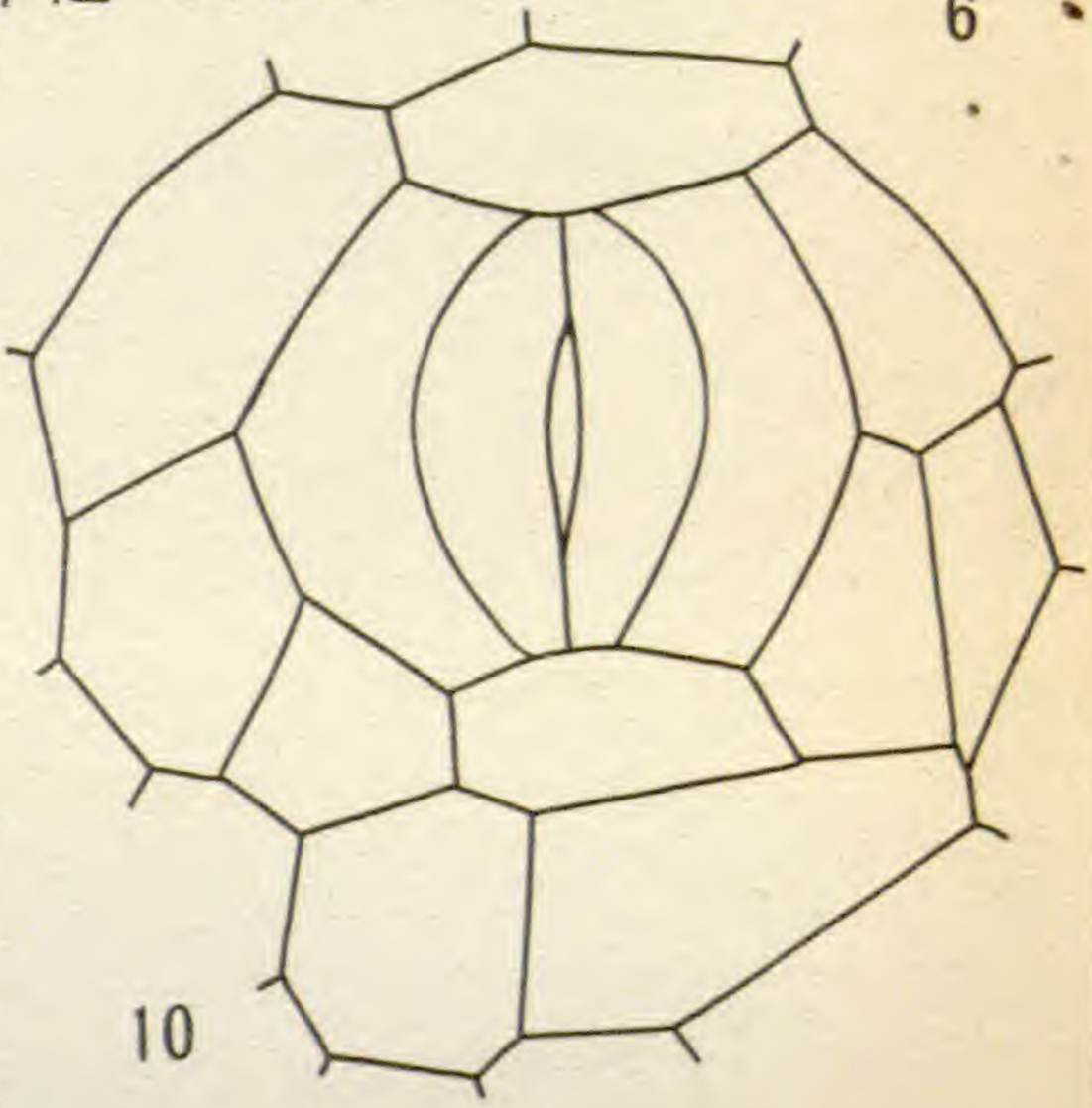

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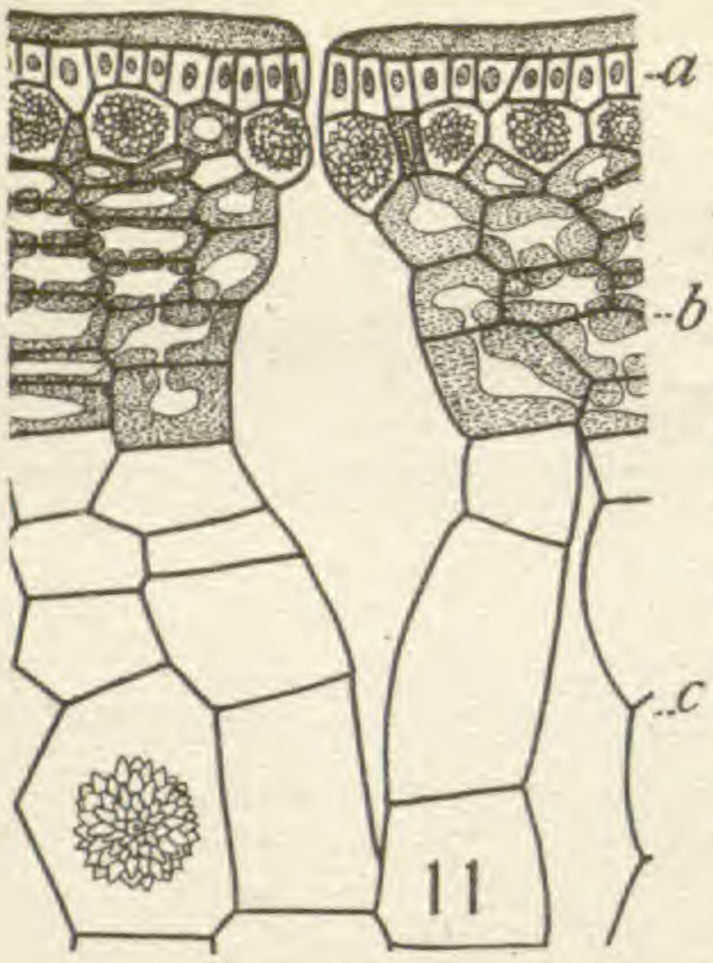
BROWN on OPUNTIA



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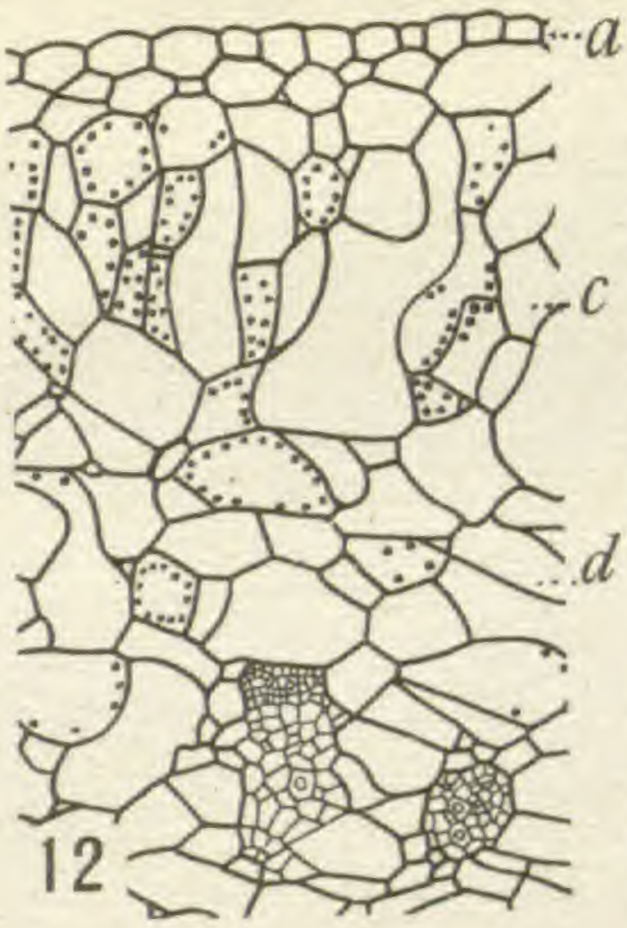

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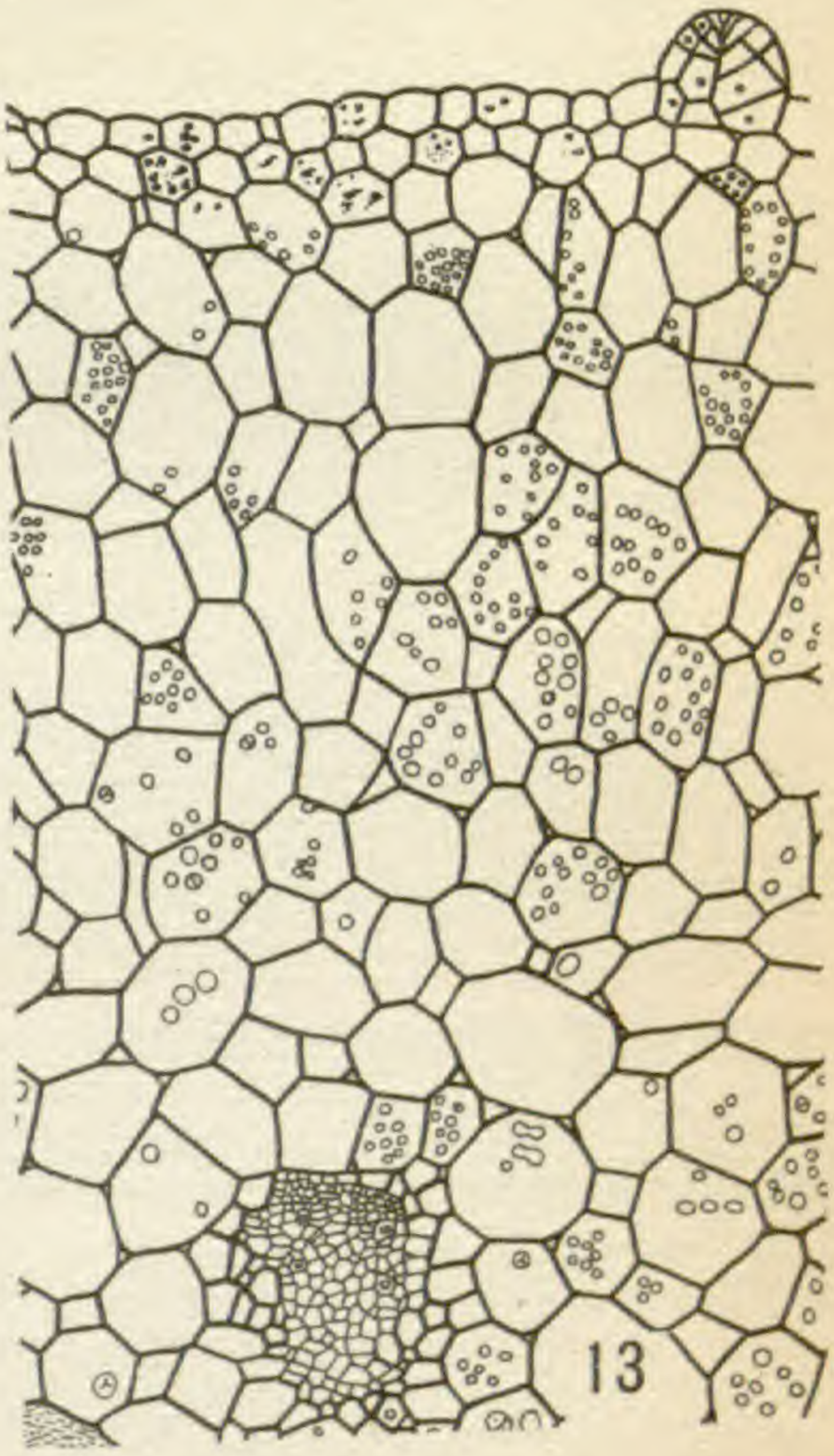

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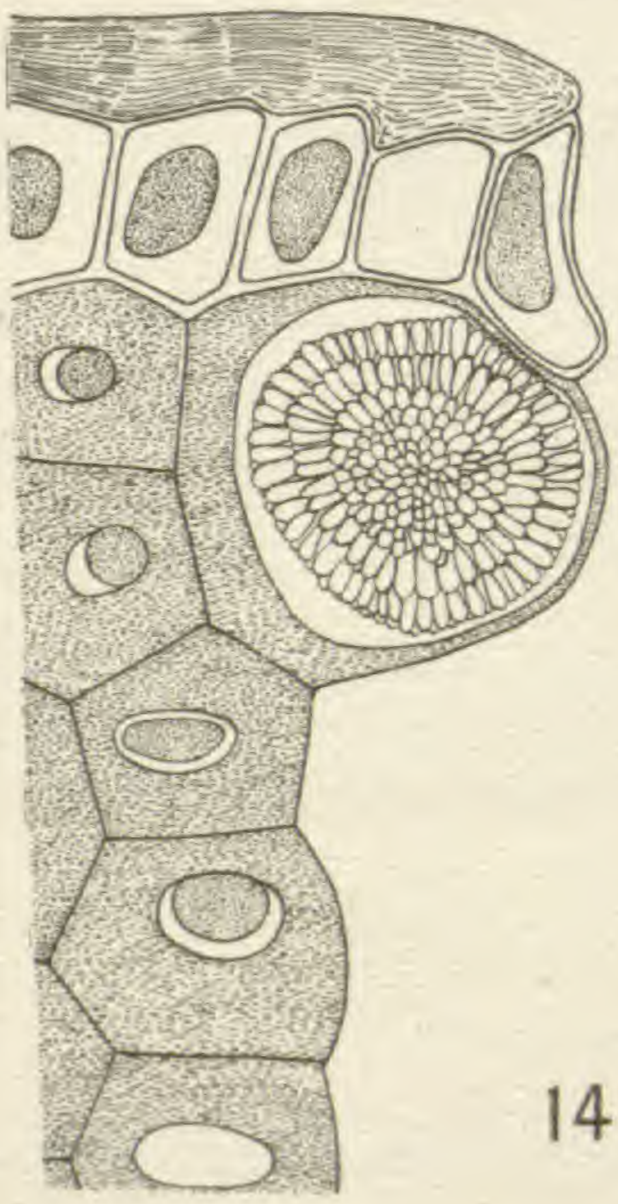

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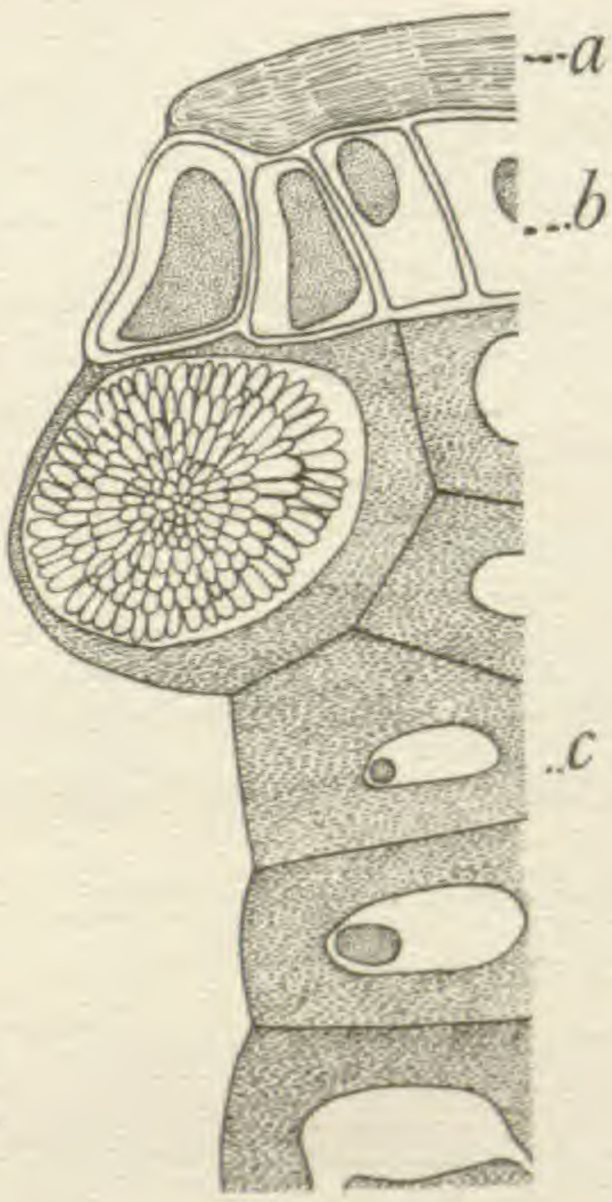

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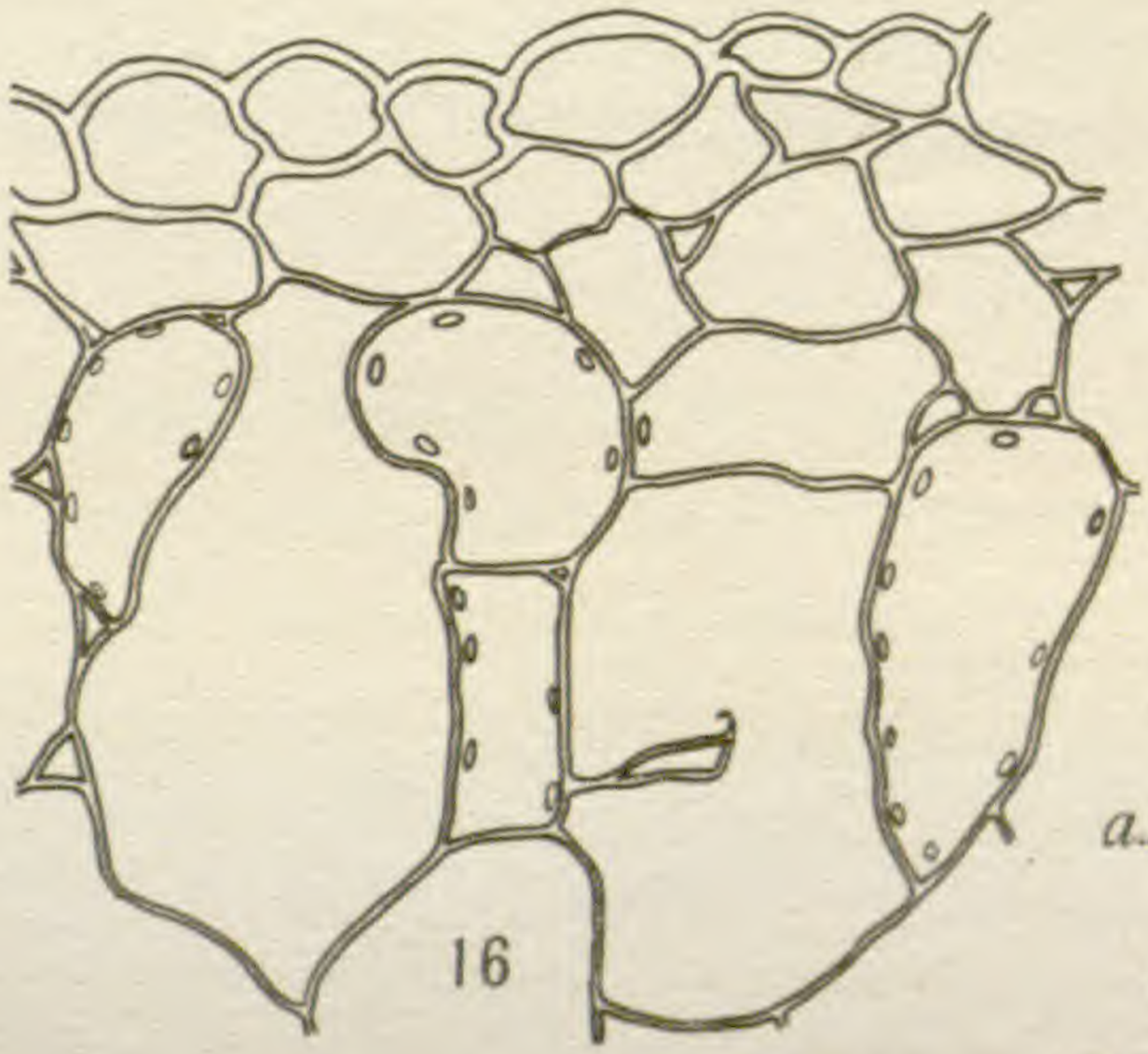

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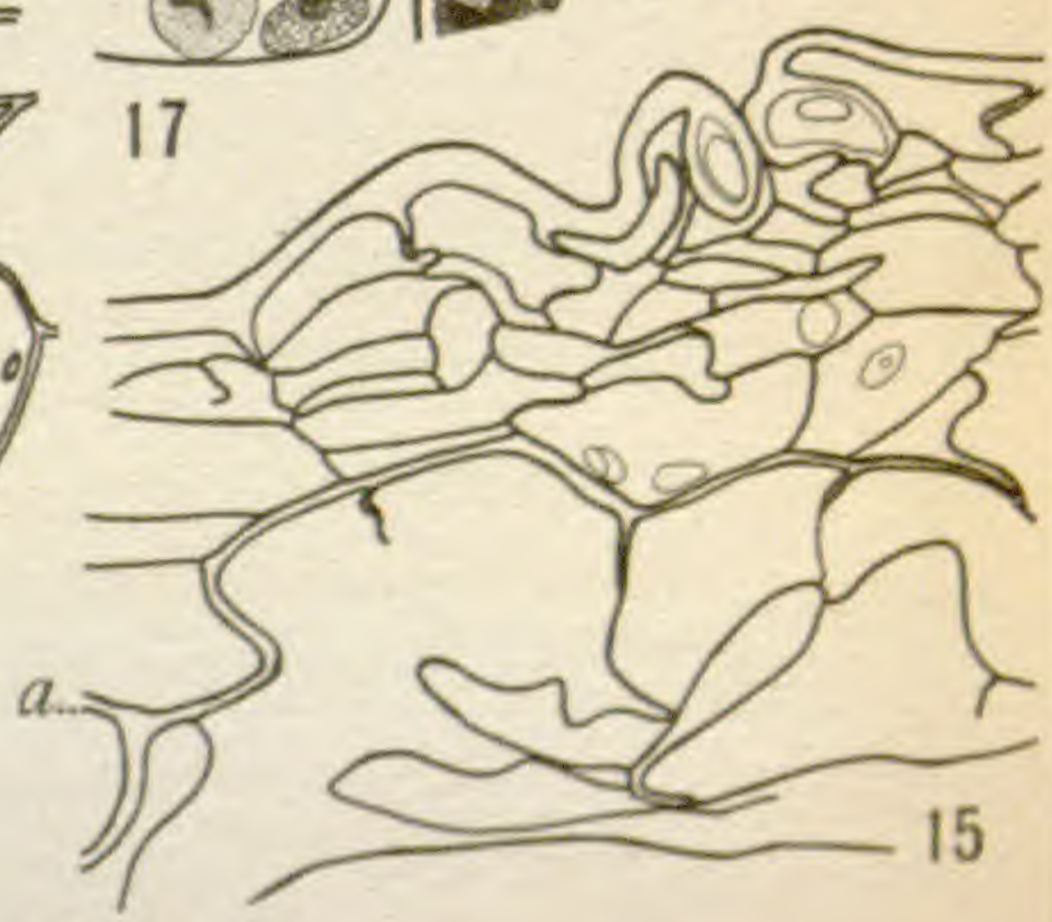

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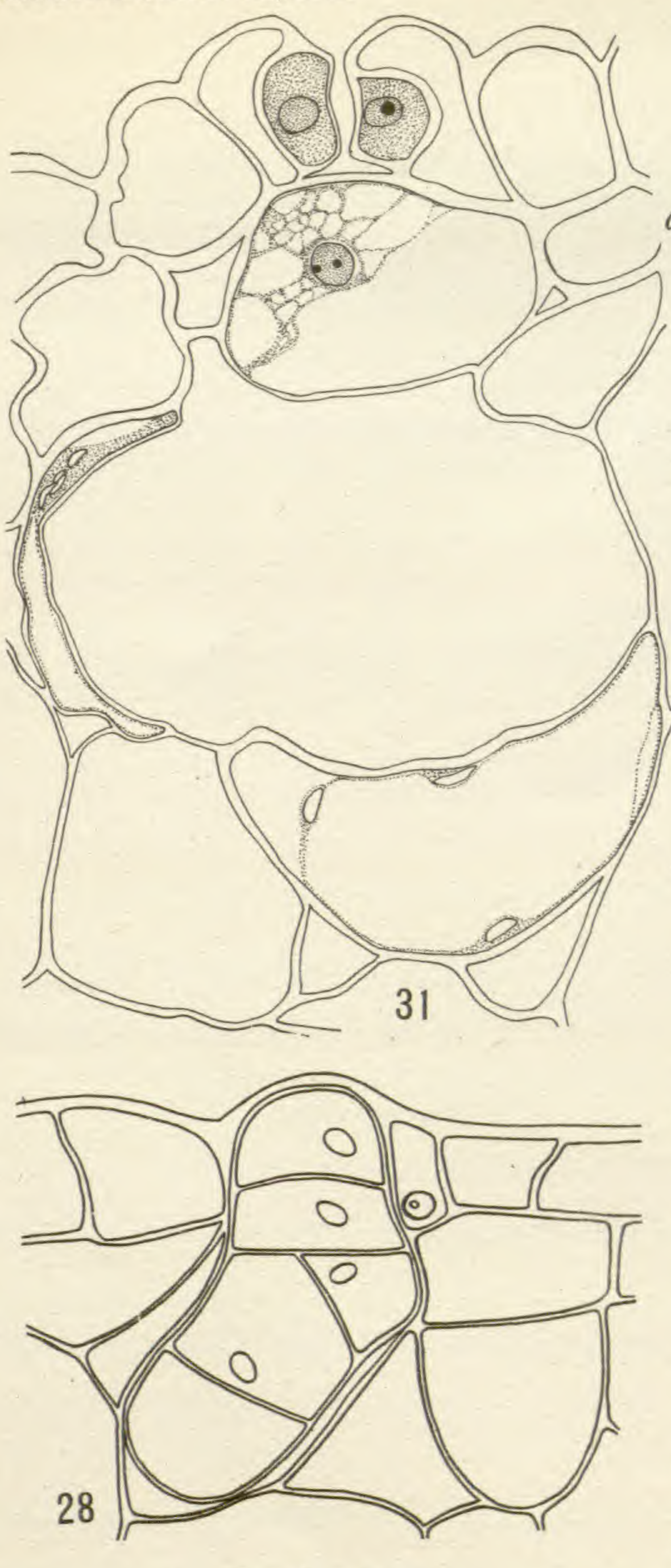
BROWN on OPUNTIA

BROWN on OPUNTIA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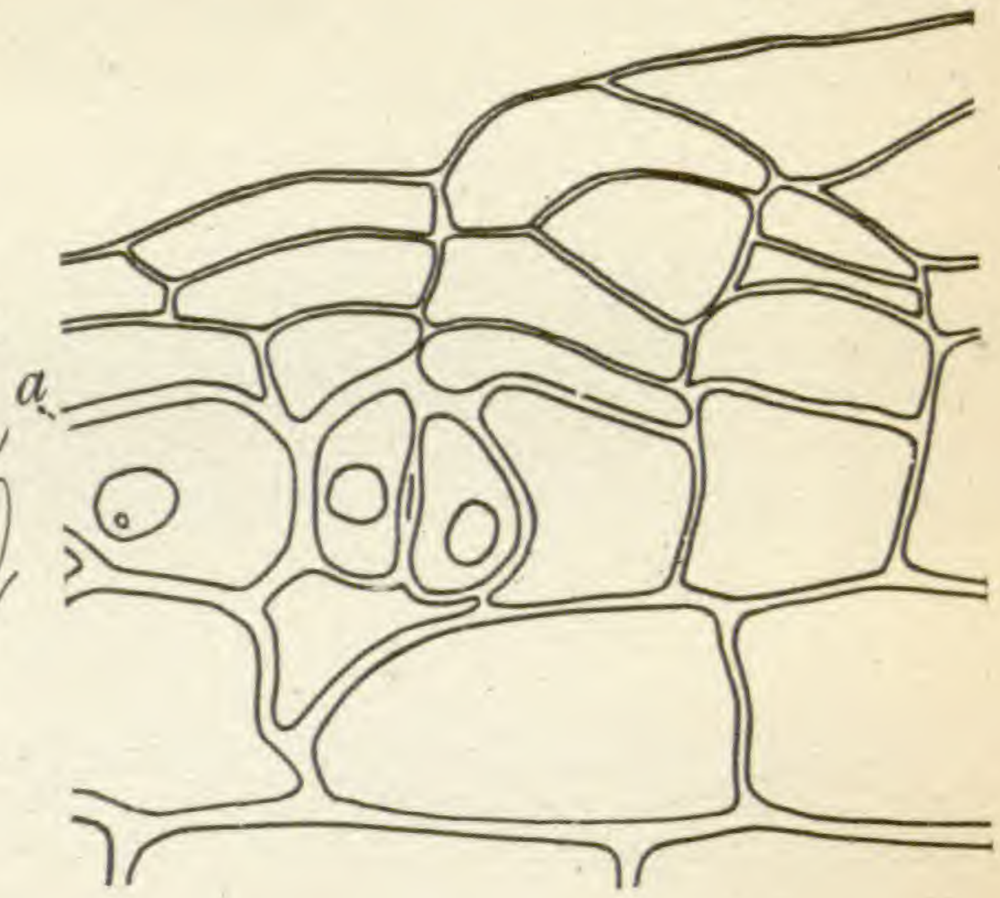
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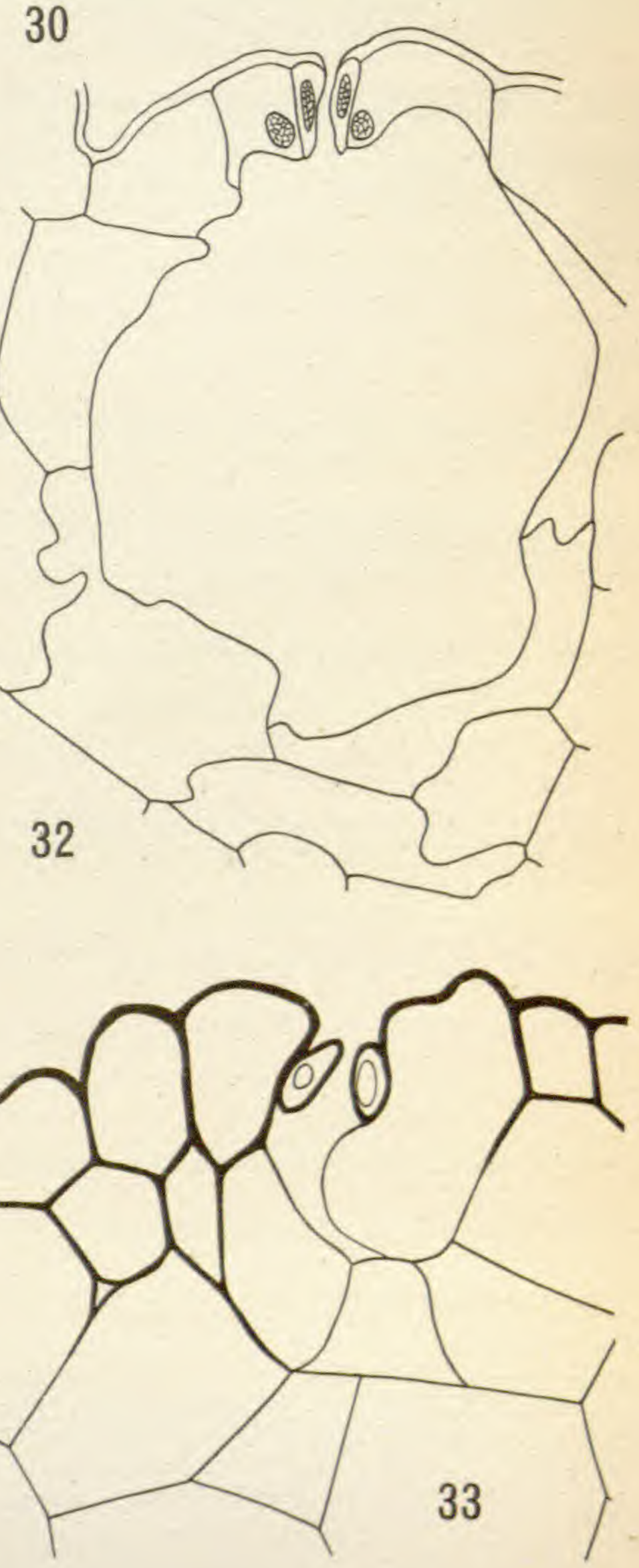
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PLATE XXX

FIG. 27.—Surface view of papilla with central stoma; ×525.

FIG. 28.—Structure found in cross-section of etiolated shoot arising like papilla, from epidermis, but growing inward; ×525.

FIG. 29.—Mass of decorticated tissue including papilla, drawn from transverse section of etiolated-greened shoot; ×525.

FIG. 30.—Subcortical development of stoma drawn from transverse section of etiolated shoot: *a*, future external epidermal wall; ×525.

FIG. 31.—Part of transverse section of etiolated-greened shoot showing stoma not yet connected with air chamber; collapsed cortical cell also appears to left; ×525.

FIG. 32.—Part of transverse section through etiolated-greened shoot: stoma lacked auxiliary cells and opened into air chamber of considerable size; similar simple stomata present in etiolated shoot; ×525.

FIG. 33.—Freakish stoma found in transverse section of etiolated shoot; ×395.

COMPOSITION OF GASES IN INTERCELLULAR SPACES OF APPLES AND POTATOES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 271

J. R. MAGNESS

(WITH ONE FIGURE)

Introduction

During a study of the ripening processes in fruits, and the chemical and physiological changes associated with them, the question has arisen as to what may be the composition of the gas in the intercellular spaces. The gas within the tissues constitutes in part the medium in which the processes associated with the life of an organism take place. It is only reasonable to suppose that the composition of this medium may exert some influence upon the rate or nature of the changes taking place. The difficulty of extracting the gases from the interior of the tissues is probably responsible for the fact that plant physiologists have almost entirely neglected studies along this line. GERBER (5) reports work of FREMY published in 1840 and 1860, in which the gas contained in apples was analyzed at intervals during their development and ripening. He found oxygen more abundant in the green fruit, the amount decreasing as the fruit matured on the tree. We have, however, no critical studies upon the internal gases of plant tissues.

An apparatus has been devised for obtaining a sample of the gas from within the tissues, without contamination with air. It is the purpose of this preliminary report to describe the apparatus and methods of sampling, together with the data secured, in order that they may be available to workers along related lines.

Apparatus

The apparatus used in extracting the gas is shown in fig. 1. It consists of a leveling bottle or burette (*A*), connected through heavy walled rubber tubing to a side neck at the base of a thick-walled glass cylinder (*B*). This cylinder is flared at the top, and

fitted with a ground glass stopper (*C*) in which is sealed a capillary tube. The flare in the top of the cylinder should be sufficient to allow the stopper to set well down (as illustrated), in order to permit a mercury seal above the stopper. The capillary tubing is bent around in the manner shown, in such a way that the tip can be immersed in a vessel of mercury (*D*). Glass stopcocks (*E* and *F*) must be in the positions shown. It is especially important that the cock *F* be as indicated, rather than directly above the cylinder, for by the former arrangement any small leak about the cock can quickly be detected. Cocks and stopper should be kept well

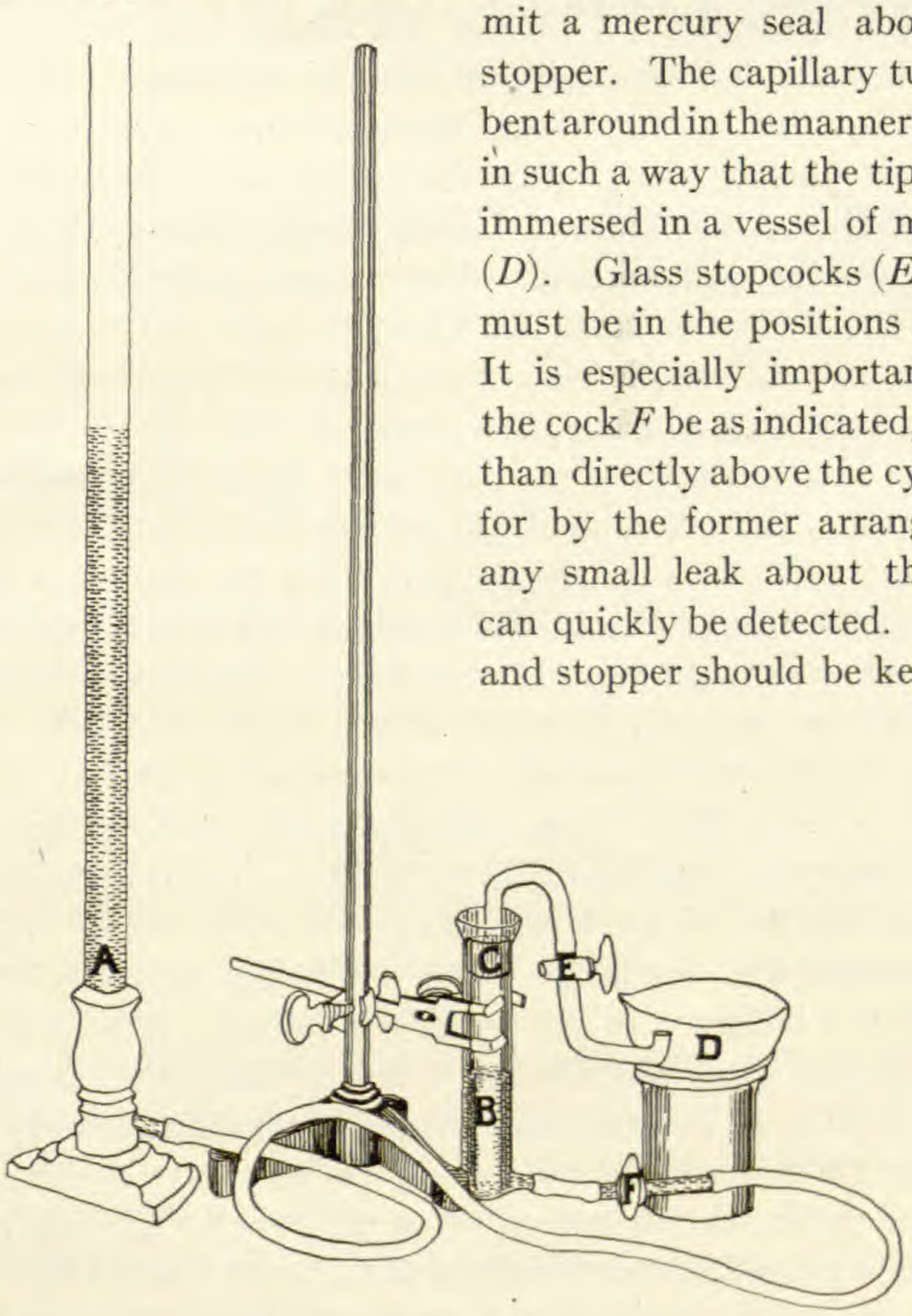

FIG. 1

coated with heavy desiccator grease. The proper dimensions for the cylinder (*B*) will obviously vary with the type of tissue being examined, and the volume of this tissue necessary to secure a gas sample adequate for an analysis. For work with apple and potato tissue, a cylinder 6 inches long and 1 inch inside diameter has been found very satisfactory.

Method

By opening the stopcock *E* and *F*, mercury was allowed to flow from the leveling burette into the cylinder until the latter was about two-thirds full. A plug of the tissue from which the gas was to be extracted was then cut with a cork borer. By using a sharp borer and cutting clean, the epidermal layers sealed each end of the plug of tissue, while all cut surfaces were in close contact with the walls of the cork borer. Consequently, there was no opportunity for contamination of the sample with the air.

The tip of the cork borer was then put under the mercury in the cylinder, and the sample plug of tissue pushed out under the mercury; a long glass rod has been found satisfactory for this. The plug was held beneath the mercury while the rod was replaced by a wire spring, and the ground glass stopper fitted into the cylinder. A mercury seal above the stopper precluded the possibility of leaks. The stopcocks (*E* and *F*) were again opened, and all air in the cylinder and capillary tubing replaced by mercury. The tip of the capillary was immersed under mercury in *D*, the cock (*E*) closed, and the leveling burette (*A*) lowered. The partial or almost complete vacuum in the cylinder causes the gas in the tissue to expand; it escapes from the tissue and collects in the top of the cylinder. The first few bubbles of gas were always discarded by driving them out through the capillary. When sufficient gas for the final analysis had collected in the top of the cylinder, it was driven off and collected over mercury in a small vial. The sample was transferred to a Bonnier-Mangin gas analysis apparatus of the type described originally by AUBERT (**2**) and later by GRAFE (**4**). CO_2 absorption was by means of 15 per cent KOH; oxygen was absorbed by 8 per cent pyrogallic acid in 30 per cent KOH. About one-half cc. of gas is sufficient for an analysis in this apparatus.

Results

Three boxes of Yellow Newton apples, representing three different trees at Watsonville, California, were used in the analyses of gas in apples. Some apples from each box were stored in a refrigerator at 6° C. and at 11° C. Others were stored in a vessel immersed in a water bath held at 20° C.; while a fourth lot was

held in an oven at 30° C. A few were held also at 2° C. In all cases abundant aeration was provided to prevent the possibility of an accumulation of CO_2 in the air surrounding the fruit. A summary of the data on the internal atmospheres in apples is given in table I.

TABLE I

ANALYSES OF GAS IN INTERCELLULAR SPACES OF YELLOW NEWTON APPLES

Temperature of storage °C.	Number of determinations	Percentage CO_2	Percentage O_2	Percentage CO_2+O_2	Percentage N_2 by difference
2.....	5	6.7	14.2	20.9	79.1
6.....	30	8.4	12.9	21.3	78.7
11.....	27	12.2	10.7	22.9	77.1
20.....	31	17.2	5.5	22.7	77.3
30.....	29	21.4	3.2	24.6	75.4

The data presented in table I require but little discussion. It is apparent that the percentage of CO_2 in the gas within the tissues increases markedly at the higher temperatures. At the same time there is a corresponding decrease in the percentage of oxygen present, the average ranging from 14.2 per cent at 2° C. to only 3.2 per cent at 30° C. These data, representing averages of a number of determinations, clearly indicate the marked variation that may occur in the composition of gas in the tissues under varying conditions of temperature.

It is of interest to note that at the lower temperatures the total percentage of oxygen plus that of carbon dioxide is about equal to that of the air. At the higher temperatures, however, and in association with the decreasing amounts of oxygen in the tissues, the sum of these two gases gradually increases. This would indicate that at the higher temperatures one molecule of oxygen liberates more than one molecule of CO_2. Such data accord with the work of GERBER (5), who found that in fleshy fruits stored at high temperatures acids were mainly respired, and that the ratio of CO_2 to O_2 under these conditions was considerably superior to unity. There is also the possibility that at the higher temperatures a certain amount of anaerobic respiration is going on, due to the relatively small amount of oxygen present. This would result in

an increased amount of CO_2 in the tissues, without a corresponding decrease in oxygen.

Table II gives the data obtained for potatoes. The potatoes used were purchased on the open market, and the variety was not determined. They were sound, smooth, and of uniform average size. A few carrots were also studied, for comparison with the apples and potatoes. From the data presented it is apparent that the same general tendency holds in potatoes and carrots that was noted in apples, that is, an increasing percentage of CO_2 and a decreasing oxygen content at higher temperatures.

TABLE II

ANALYSES OF GAS IN INTERCELLULAR SPACES OF POTATOES AND CARROTS

Temperature of storage °C.	Number of determinations	Percentage CO_2	Percentage O_2	Percentage CO_2+O_2	Percentage N_2 by difference
	Potatoes				
11	8	19.6	10.9	30.5	69.5
22	8	34.4	5.7	40.1	59.9
	Carrots				
11	2	12.2	13.1	25.3	76.7
24	2	28.6	5.2	33.8	66.2

It will be noted that the total CO_2 and oxygen is much higher in the case of potatoes than was found in apples. This variation may be due in part to the fact that there is relatively much less intercellular space in potatoes than in apples, and a higher percentage of the gas may have come out of solution in the juice in the samples obtained from potatoes than in those obtained from apples. It is necessary to use much larger samples of potato tissue in order to obtain sufficient gas for an analysis than is essential when apple tissue is used.

The amount of gas that may be coming out of solution in the juice, rather than from the intercellular spaces, presents a difficulty inherent in this method of sampling. There is no assurance that the gas that comes out of solution is of exactly the same composition as that of the intercellular spaces. The consistent results recorded

for fruit under the different temperatures tested, however, clearly indicate the tendency of the oxygen-carbon dioxide ratio within the tissues, regardless of the fact that the absolute values may vary somewhat.

Effect of wounding

Many references to the effect of wounding plant tissues upon rate of respiration are found in the literature. Invariably wounding of the tissue has resulted in an increased rate of respiration. GERBER has found this to be true of apples, grapes, and other fruits. APPLEMAN (1) has reported the same phenomenon for white potatoes.

A few apples were prepared for a study of the effect of wounding upon the composition of the internal atmosphere. A thin slice of the peel was removed from each end of the fruits, and they were then put in storage at the various temperatures by the side of whole fruits serving as checks. The data from the analyses of these fruits are reported in table III. It is apparent from these data that removing the epidermis greatly facilitates the entrance of oxygen to the tissues, and also the escape of accumulated CO_2. It would be interesting to know to what extent increased respiration following wounding is due to mechanically facilitating this gaseous exchange, and to what extent it is due to actual metabolic changes in the wounded tissues.

TABLE III

EFFECT OF REMOVING PEEL FROM ENDS OF FRUITS UPON COMPOSITION OF INTERNAL ATMOSPHERE

Temperature °C.	Treatment	Number of Determinations	Percentage CO_2	Percentage O_2	Percentage CO_2 + O_2
1.....	Whole apples	3	6.6	14.6	21.2
1.....	Ends peeled	4	1.7	15.8	17.5
20.....	Whole apples	2	17.8	7.0	24.8
20.....	Ends peeled	2	7.4	9.9	17.3
30.....	Whole apples	2	23.9	1.8	25.7
30.....	Ends peeled	2	12.6	8.9	21.5

Variation in composition of gases

Considerable variation occurred between individual apples or potatoes held under identical conditions. This is to be expected

when the wide variation in size, thickness of epidermis, etc., is considered. The extremes of variation found in apples held at 20° C. are indicative of the range of fluctuation that may be encountered in work of this type. The 31 apples analyzed at this temperature contained gas averaging 17.2 per cent CO_2. The minimum CO_2 recorded for any apple of the lot was 12.5 per cent; the maximum 25.7 per cent. Only one apple, however, showed more than 21.8 per cent, so that this latter figure is a more accurate maximum. The extremes of oxygen variation were somewhat less. With an average of 5.5 per cent oxygen, the minimum value was 1.0 per cent, and the maximum 9.5 per cent. Although most of the values were very much nearer the mean than these, it is essential that a considerable number of individual analyses be made to determine the true mean for any given condition.

Factors influencing amount

Three main factors operate to determine the amounts of CO_2 and oxygen in the intercellular spaces at any given temperature. These are (1) the rate of oxidation, or the rate at which oxygen is taken up from and CO_2 given off into the intercellular spaces; (2) the permeability of the skin or epidermal covering to CO_2 and oxygen; and (3) the difference in pressure of CO_2 and oxygen within and without the fruit, which determines the rate of gaseous exchange when the permeability factor is constant. The effect on each of these factors of varying the temperature will explain the variation occurring in the internal atmosphere of the tissues studied at the different temperatures.

EFFECT OF TEMPERATURE ON OXIDATION PROCESSES.—GORE (6) has found that the rate of respiration for a large number of fruits, as measured by the quantity of CO_2 given off, increased, on an average, 2.38 times for a 10° rise in temperature. Enzymatic processes in general, within the range of temperatures here studied, show an increase of from two to three times for each 10° rise. It is thus apparent that the oxidative processes will be speeded up very markedly by temperature increases.

EFFECT OF TEMPERATURE ON PERMEABILITY.—DENNY (3), in a study of the permeability of a number of plant membranes, has

found that in general the increase in permeability per 10° rise in temperature varies from 1.3 to 1.8 times, averaging about 1.5. These data are based on permeability to water, but there is no reason for believing that gases would be fundamentally different. The diffusion of gases in all probability is mainly a physical process, and as such is relatively much less affected by temperature changes than the chemical changes involved in oxidation.

From a consideration of these relative effects of temperature on oxidation and on permeability, it is apparent that the absorption of oxygen and release of CO_2 are increased much more by a given rise in temperature than is the tendency for oxygen to be supplied to the tissues, and CO_2 to be given off from them. Consequently, as the temperature is raised, the amount of oxygen in the tissues becomes less and less, while the CO_2 accumulates correspondingly. This continues until the third factor becomes effective, that is, the difference of CO_2 and oxygen pressures within and without the fruit becomes so great that equilibrium is again established.

Significance of ratio

No attempt has been made in this preliminary work to associate the percentages of CO_2 and oxygen found with the processes taking place in the fruit. The data presented, however, clearly indicate the necessity of taking this factor into consideration in many types of horticultural and physiological investigations. It should be given attention in studies of the effect of temperature upon the processes in plant tissues, for it is readily apparent that much variation may be caused by the composition of the medium in which these processes are carried on. Of special importance is the application of studies of this type to the questions as to the effect of wounding and various other treatments on the respiratory processes in tissues. Finally, it is of prime importance to know the composition of the internal atmosphere in studying the effects of various gases, etc., on plant organs. Some work has been done on the effect of various gases on fruits and vegetables in storage. Obviously it is essential in such work that the composition of the internal atmosphere be known.

The writer feels deeply indebted to Dr. WILLIAM CROCKER, and to Mr. W. S. BALLARD, U.S. Department of Agriculture, for valuable suggestions in regard to the apparatus for extracting the gas, and for many helpful suggestions during the progress of this work.

U.S. DEPARTMENT OF AGRICULTURE
WATSONVILLE, CAL.

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BRIEFER ARTICLES

USE OF DILATOMETER IN STUDYING SOIL AND PLANT RELATIONSHIPS

The dilatometer was used successfully by BOUYOUCOS[1] in studying the forms of water in soils, and in studying the freezing point lowerings of soils and plants, MCCOOL and MILLAR[2] observed that the time of day when samples of plants were taken markedly influenced the freezing point lowerings of the leaves. The density of the sap was found to increase from morning until noon, and again decrease in the afternoon, reaching its lowest point at night. By means of the dilatometer the amount of water that froze readily was less at noon than in the morning, the freezing point lowerings apparently being governed somewhat by the form of water in the tissues.

We have repeated some of the experiments that were previously reported, and obtained additional information by means of the dilatometer. There are certain precautions that should be taken in making determinations of the amount of water that freezes in plant tissues by means of the dilatometer.

The sample is quickly weighed, inserted into the bowl of the dilatometer, and ligroin added. It is advisable to remove air bubbles either by shaking or by means of a suction pump. Where a bath of about $-1.5°$ C. is employed, 10 gm. of the tissue may be used; but with colder baths, such as -4 or $-6°$ C., it is imperative that much smaller quantities of tissue be added to the dilatometer, in order that supercooling may be brought about. With some plants 2 gm. are ample, while with others somewhat larger amounts may be employed to advantage. It is very difficult to accomplish supercooling if the freezing mixture or the dilatometer is agitated while the temperature of the material is being lowered. When equilibrium has been attained, solidification may be accomplished readily by agitating the dilatometer.

Several plants have been employed, namely, rye, wheat, corn, sweet clover, and red clover. They were grown on fertilized and untreated

[1] Tech. Bull. no. 36, Mich. Exper. Sta.

[2] The water content of the soil and the composition and concentration of the soil solution as indicated by the freezing point lowerings of the roots and tops of plants. Soil Sci. 3:113–138. 1917.

soils. The amounts of water that froze at different temperatures are reported in table I.

TABLE I

AMOUNT OF WATER FREEZING IN LEAVES OF PLANTS AT DIFFERENT TEMPERATURES

Crop	Date	Weight of material (gm.)	Freezing point lowering	Cc. water that froze		
				−1.5° C.	−4° C.	−6° C.
Rye.....	Nov. 24	5	0.928	0.90	2.50	.....
Rye.....	May 17	5	1.030	0.86	2.40	2.90
Wheat.....	Nov. 24	5	1.107	0.40	2.65	.....
Sweet clover.	Nov. 24	5	0.906	1.22	2.86	.....
Red clover...	May 15	5	0.780	1.70	2.70	2.90
Corn.....	June 10	5	0.578	2.10	2.40	.....

Marked variations in the amount of water that froze at −1.5° C. were observed, and while the amount increased with the lower temperatures, less differences were recorded.

The effect of the concentration of the soil solution and the water content of the soil were determined. The density of the solution in the soil was increased and so maintained by additions of the three salt solutions of SHIVE and the soil placed in 3 gallon jars and plants grown therein. The containers were placed in the open and exposed except when it rained.

Marked increase in the density of the soil solution resulted in little if any effect on the amount of water that froze at −2.5° and −4° C. respectively. The results are not given, inasmuch as MILLAR is to report them in another article.

The amount of water in the soil affected the quantity of water that froze in the plants grown therein. Corn was grown 60 days in sandy loam soil containing 9.5 and 15.6 per cent water respectively; 44.1 per cent of the loss in weight of the corn upon drying, froze at −2.5° C., and 63.9 per cent at −4° C. in the former and 51.8 and 84.8 per cent respectively in the latter. Similar results were obtained with barley.

In another series the moisture content of the soil was varied, but the concentration of the soil solution was kept about the same throughout the experiment by additions of the nutrient solution. In case of the higher temperature the results obtained were the reverse of those just given, or the amount of water that froze in the corn plant increased with a decrease in the water content of the soil when the nutrients were added. Slight differences were observed when the tissue was exposed to a temperature of −4.5° C.

This raises the question as to the effect of the composition of the soil solution upon the amount of easily freezable water in plant tissue. Possibly we shall be able to present results of experiments dealing with this question at a later date. It seems that the amount of water that readily freezes in the roots, stems, leaves, fruits, and seeds of plants and the factors that affect the freezing should be of general interest, at least to the physiologist, and it is probable that a knowledge of it would be valuable especially where the changes in the concentration of the cell contents of plants as well as winter injury are being investigated.

The difficulty encountered in causing tissues to solidify at the higher temperatures, especially when small amounts are used in the determinations, raises some important questions relative to winter injury of plants grown in different soils. It is possible and probable that some soils do not solidify, although the temperature may go appreciably below the freezing point. It is very easy to cause a sandy soil to solidify without much supercooling; with clay it is more difficult; while it is far more difficult in case of muck or peat. Instances have been observed where fruit trees growing in sandy soils have been severely injured by low temperature, while those growing in adjacent soils largely escaped. It is true, however, that sandy soils are more responsive to air changes than are the finer textured ones.—M. M. McCool and C. E. Millar, *Michigan Agricultural College, East Lansing, Mich.*

ISOLATING SINGLE SPORES

(WITH ONE FIGURE)

A new method of isolating single spores has been devised, which differs from other methods in common use in the substitution of a mechanical method of marking the location of the spores in the poured plates for the usual procedure of marking with ink-dots under the microscope. A cylinder of brass about the length of the ordinary 1.9 mm. objective is turned in the form shown in fig. 1, one end being provided with a thread like that of the objectives of the microscope to be used, and the other turned down and the end hollowed out so as to form a tube of the size desired.

This device is then screwed into the revolving nosepiece of the microscope in place of one of the objectives. The cover is now removed from the Petri dish containing the poured plates, and the spores are located under the microscope. When a spore is located with the objective, the tip of the marker is sterilized by flaming it with a gas burner or alcohol lamp, the nosepiece is rotated so as to bring the marker

over the spore in the place of the objective, and the marker is lowered so as to cut out a disk of agar inclosing the spore. The spore may then be examined again with the objective to see that it is really included in the agar disk, after which the disk is lifted from the Petri dish with a flattened platinum wire and placed in a culture tube. This method has the advantage of allowing a rapid and accurate location of spores and of guaranteeing that only a single spore is transferred. The disadvantages of exposing the culture to the air for rather long intervals is not a great one, because contaminations need not occur if proper precautions are taken. For such work the ringed tip of the marker may have a diameter of about 5 mm.

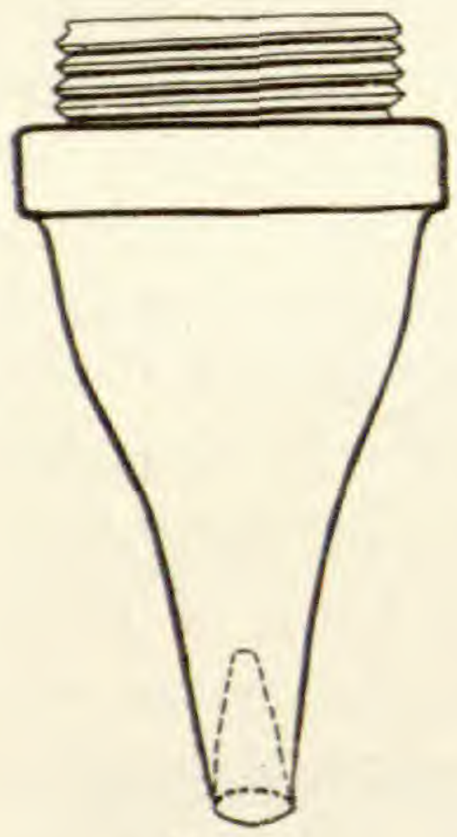

FIG. 1

A modification of this method has been found of great use in the selection of spores of *Pestalozzia*. In this case it was necessary, not only to locate single spores accurately, but also to measure the spores when located, so that the longest and the shortest might be taken. Finally it was found that this could be done by making dilution cultures, and then spreading the agar of the tube containing the desired dilution in a thin film on sterile glass slides. These slides could then be examined under the microscope, the spores measured, and those of the size desired cut out by the marking device, and finally the disks containing the spores could be transferred to culture tubes.

The agar for making the dilution cultures needs to be very carefully filtered so as to be as transparent as possible. For the work with *Pestalozzia* a 1 per cent solution of Liebig's beef extract with 3 per cent agar was used and found satisfactory. The nutrient solution, of course, must be of such a nature as to be suited to the fungus in question. The agar film on the slides is formed by pouring two or three drops of agar on the slide and then spreading them over the entire surface with a sterile needle. Too thin a film cannot be lifted from the slide; too thick a film will not allow the high power objective to be used, which is necessary with measurements of very small spores, so that some practice is needed to secure good results. I am indebted to Miss BACHMANN[3] for the idea of using agar films on slides, although films formed according to her method are too thin for this purpose. In this selection work a marker with a tip 1.5 mm. in diameter was found most useful.—CARL D. LARUE, *Kisaran, Asahan, Sumatra.*

[3] BACHMANN, FREDA M., Amer. Jour. Bot. **5**:32–35. 1918.

CURRENT LITERATURE

NOTES FOR STUDENTS

Taxonomic notes.—BECCARI[1] has monographed the palms of the Philippine Islands, recognizing 129 species in 22 genera, besides numerous varieties. Only 15 of these species have a wide distribution, and 5 are naturalized, leaving 109 endemic species. The new species are 10 in number, and *Adonidia* is established as a new genus. Much the largest genus is *Calamus*, with 36 species.

EVANS[2] has monographed the North American representatives of *Asterella*, "a difficult group of liverworts." A discussion of the morphological features of the genus is followed by a detailed taxonomic treatment of the 14 species, 3 of which are new.

PENNELL[3] has begun a series of papers dealing with the Scrophulariaceae of Wyoming, Colorado, and Utah, and of Idaho west to the 113th meridian, based upon extensive field work and also upon critical study of herbarium material. The purpose is to give for each species its taxonomic history, its flowering season, and its distribution. The first paper deals chiefly with *Pentstemon*, in which 88 species are recognized and arranged under 19 sections, 27 of the species being described as new.

BRAUSE,[4] in his study of Papuan Pteridophytes, describes 45 new species, chiefly in *Polypodium* (19), *Vittaria* (6), *Angiopteris* (5), and *Selaginella* (10). Of the 970 species of Pteridophytes listed, 597 are said to be endemic.

BROTHERUS,[5] in describing WEBERBAUER's collection of South American mosses, describes 29 new species.

PILGER,[6] in continuing his studies of South American grasses, describes 9 new species.

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[1] BECCARI, O., The palms of the Philippine Islands. Philippine Jour. Sci. **14**: 295–362. *pls. 3.* 1919.

[2] EVANS, A. W., The North American species of *Asterella*. Contrib. U.S. Nat. Herb. **20**:247–312. 1920.

[3] PENNELL, F. W., Scrophulariaceae of the Central Rocky Mountain States. Contrib. U.S. Nat. Herb. **20**:313–381. 1920.

[4] BRAUSE, G., Bearbeitung der von C. LEDERMANN von der Sepik-Expedition 1912 bis 1913 und von anderen Sammlern aus dem Papuagebiete früher mitgebrachten Pteridophyten, nebst Übersicht über alle bis jetzt aus dem Papuagebiet bekannt gewordenen Arten derselben. Bot. Jahrb. **56**:161–250. 1920.

[5] BROTHERUS, V. F., Musci Weberbaueriani. Beibl. Bot. Jahrb. **56**:1–22. 1920.

[6] PILGER, R., Gramineae austro-americanae imprimis Weberbauerianae. V. Beibl. Bot. Jahrb. **56**:23–30. 1920.

RIDLEY[7] has described two new genera of Malayan plants, namely *Peripetasma* (Menispermaceae) and *Scaphocalyx* (Flacourtiaceae).

MINOD[8] has made a very detailed study of the American species of *Stemodia* (Scrophulariaceae), recognizing 30 species, 5 of which are described as new. Thirteen species are eliminated from the genus, and in this connection the following new genera are established: *Chodaphyton*, *Lendneria*, *Verena*, and *Valeria*.

SMITH[9] has segregated a new genus (*Whytockia*) from *Stauranthera*, naming it for Mr. JAMES WHYTOCK, president of the Botanical Society of Edinburgh. It occurs in mountain forests of western China (Yunnan). The statement is made that recent collections show that southern and western China is rich in Gesneraceae.

Miss CURRIE[10] has published a critical study of Myxomycetes, chiefly from Ontario, representing 117 species and varieties, included in 29 genera. In several cases she has uncovered new facts as to morphology, physiology, and distribution. In the list of species and varieties are included 47 not previously recorded from Ontario, 36 new to Canada, and 5 new to North America. The plasmodia of two species were observed for the first time, a number are recorded as parasitizing fungi, and others as fruiting on leaves or stems of grasses or herbs to which they are injurious in some cases. The contribution represents a valuable addition to our knowledge of this interesting group.

HITCHCOCK and Mrs. CHASE,[11] in continuation of their studies of North American grasses, have published a revision of four genera of Paniceae, with full descriptions, details of distribution, and analytical keys. *Ichnanthus* is tropical American and includes 10 species, 5 of which have been published recently by the authors. *Lasiacis* is also tropical American, one species extending into Southern Florida. It was segregated from *Panicum* some years ago by HITCHCOCK, and is now recognized to include 15 species, one of which is described as new. *Brachiaria* extends into the southern United States and includes 5 species, one of which is new. *Cenchrus*, widely distributed, includes 13 species. The first two genera are revised by HITCHCOCK, and the last two by Mrs. CHASE.—J. M. C.

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[7] RIDLEY, H. N., New Malayan plants. Jour. Botany **58**:147–149. 1920.

[8] MINOD, MARCEL, Contribution a l'étude du genre *Stemodia* et du groupe des Stémodiées en Amérique. Thèse no. 606. Institut de Botanique, Univ. Genève. pp. 103. *figs. 41*. 1918.

[9] SMITH, W. W., *Whytockia*, a new genus of Gesneraceae. Trans. and Proc. Bot. Soc. Edinburgh **27**:338, 339. *pl. 7*. 1919.

[10] CURRIE, MARY E., A critical study of the slime-molds of Ontario. Trans. Roy. Can. Inst. **12**:247–308. *pls. 8–10*. 1919.

[11] HITCHCOCK, A. S., and CHASE, AGNES, Revisions of North American grasses. Contrib. U.S. Nat. Herb. **22**:1–71. *pls. 24. figs. 20*. 1920.

Chemical stimulation.—STEINBERG[12] has reported on further studies on the chemical stimulation of growth in *Aspergillus niger*, especially as brought about by Zn and Fe salts. Pfeffer's nutrient solution was used as the basis of all cultures. Commercial reagents, without further purification, were used in making solutions. It was found that 0.1 to 1.0 mg. Zn per liter was sufficient to furnish maximum stimulation, the growth thus stimulated averaging about four times that of the check. Successively lessening the amounts of each of the inorganic constituents of the solution resulted in all cases in decreased yield, showing none of them present in toxic or super-optimum concentration. Increasing the concentration of the various salts stimulated growth, although to a much less extent than that caused by Zn. Decreasing the acidity by adding alkalis decreased growth and gave strong spore formation. Increasing the H ion concentration by adding various acids stimulated vegetative growth and decreased spore formation. The type of growth resulting from increasing H ion concentration was very similar to that following Zn stimulation, but was always less in amount. STEINBERG believes the H ion concentration of Pfeffer's solution to be sub-optimum for *A. niger*, and suggests that the increased acidity of the solution with the addition of salts of the heavy metals with strong acids may be a very important factor in the stimulation to greater growth.

The Pfeffer solution was purified by autoclaving with $CaCO_3$ to precipitate Zn and other bivalent metals. Growth in such solutions was much less than in those not purified. Either Zn or Fe added alone stimulated growth somewhat, but not nearly so much as when both were added. Growth in the latter case was equal to that in any of the non-purified Zn stimulated cultures. STEINBERG believes previously secured stimulation from Zn alone to have been in the presence of Fe impurities.

No analyses were made to determine the chemical changes associated with the marked variation in growth rate and form brought about by the variation in the nutrient solution. Such data would be most interesting. It is unfortunate that reagents were not sufficiently repurified to eliminate entirely the possibility of Zn, Fe, Ni, and other such metals in the basic nutrient solution.—J. R. MAGNESS.

Mangroves.—Among the interesting features of a study of the red mangrove, *Rhizophora Mangle*, by BOWMAN,[13] is a synopsis of the historical development of our knowledge of the tree. The literature dates from 325 B.C. to the present, and includes references by THEOPHRASTUS, PLINY, PLUTARCH, BAUHIN, RAY, LINNAEUS, and LAMARCK, as well as more recent writers.

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[12] STEINBERG, R. A., A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. Amer. Jour. Bot. **6**:330–372. 1919.

[13] BOWMAN, H. H. M., Ecology and physiology of the red mangrove. Proc. Amer. Phil. Soc. **56**:589–672. *pls. 9*. 1917.

The investigations of BOWMAN were in the Dry Tortugas, and include many details regarding the morphology and structure of the various organs of the tree, including development and growth rate of the viviparous embryos. Measurements of the latter show a 4.7 cm. elongation of the emerging hypocotyls in 34 days. The results of transpiration studies show a lower rate of water loss with higher concentrations of sea water. The red mangrove is facultative in its growth in fresh and salt water, but requires the latter for optimum development. At least 2000 sq. miles of the tidal flats of the Philippine Islands are occupied by mangrove forests. The floristic, ecological, and economic characteristics of these forests of the sea have been described by BROWN and FISCHER.[14] Keys are provided for the recognition of the 30 principal species belonging to 16 different families. In addition to the well known aerial roots and viviparous habit of the mangroves, some of the notable features of these woodlands are the scanty undergrowth, the fairly numerous epiphytes, the myrmecophilous plants, and the frequent fringing of Nipa palms.

While the original stands of this forest contain trees of fair size yielding hard cabinet woods of excellent quality, the greater portion of the area is important only for the production of a good quality of firewood and for tan bark. The Nipa palm is important for alcohol production, and seems to present a possibility of utilization for sugar. Some cultivation of both the mangroves and the Nipa palms has proved successful; the former has also been used with good results in planting dykes and embankments to prevent the erosive action of the sea.—GEO. D. FULLER.

Age and area hypothesis.—The development of this hypothesis by WILLIS has been noted in this journal,[15] and now an analysis of the flora of New Zealand seems to strengthen his contentions.[16] The evidence in favor of the majority of endemics being of recent origin rather than relics is rather convincing.

Recently a floristic study of the plants of Stewart Island[17] yielded results supporting the hypothesis of the families and genera being represented in proportion to the number of genera and species respectively contained in them in New Zealand. The oldest forms are best represented in the flora, and the endemics are in the largest (in general, oldest) families and genera of New Zealand.

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[14] BROWN, WM. H., and FISCHER, A. F., Philippine mangrove swamps. P.I. Dept. Agric. and Nat. Res., Bur. For. Bull. **17**:132. *pls. 47*. 1918.

[15] BOT. GAZ. **61**:82. 1916; **62**:160. 1916; **63**:419. 1917; **64**:263. 1917; **65**: 116–117, 486. 1918.

[16] WILLIS, J. C., The sources and distribution of the New Zealand flora, with a reply to criticism. Ann. Botany **32**:339–367. 1918.

[17] ———, The flora of Stewart Island (New Zealand): a study in taxonomic distribution. Ann. Botany **33**:23–46. 1919.

The other islands about New Zealand also supply similar data.[18] In studying these floras WILLIS contends that through this hypothesis one is able to prophesy that the plants which reach outlying islands will be on the whole the oldest, and therefore the most widespread upon the mainland, and finds, on examining the facts, that the prophecy is completely fulfilled. The facts presented seem to support the contention and lead the author to restate the hypothesis thus: "The area occupied at any given time, in any given country, by any group of allied species at least ten in number, depends chiefly, so long as the conditions remain reasonably constant, upon the age of the species of that group in that country, but may be enormously modified by the presence of barriers such as seas, rivers, mountains, change of climate from one region to the next or other ecological boundaries, and the like, also by the action of man, and by other causes. In other words, age and area is the chief positive, the action of barriers the chief negative, factor in plant distribution, while in recent times the action of man has become of greater importance than either."—GEO. D. FULLER.

Gases and germination.—KIDD[19] has studied the effect of various partial pressures of carbon dioxide and oxygen upon the sprouting of potatoes, and concludes that "(1) Oxygen is harmful to the potato tuber in concentration of about 5–10 per cent; oxygen 80 per cent kills in 4–5 weeks; oxygen 5–10 per cent is the optimal concentration for sprouting. (2) The harmful action of oxygen is increased in the presence of carbon dioxide. (3) Carbon dioxide inhibits sprouting in a concentration of 20 per cent. This concentration is at the same time to some extent harmful. (4) Higher concentrations of carbon dioxide cause marked injury and death." NOBOKIRCH has found that actively growing plant organs grow faster in oxygen pressures considerably below that of the normal atmosphere, but that such reduced pressures finally prove injurious, due to accumulation of metabolic products; while at normal oxygen pressures no such injury occurs. This may throw in question KIDD's interpretation that pressures above 10 per cent are injurious, especially for pressures up to the normal atmosphere. In general, due to their coats and other coverings, seeds are reduced in rate and percentage of germination by any reduction of oxygen pressure below the normal atmosphere, and often favored by greater oxygen pressures. Some of the work of APPLEMAN has indicated that oxygen supply is a limiting factor to germination of the potato, quite in contrast with KIDD's results. It is interesting that carbon dioxide showed no forcing action due to its anaesthetic properties. It is possible that it did cause increases in respiration, while not increasing or

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[18] WILLIS, J. C., The floras of the outlying islands of New Zealand and their distribution. Ann. Botany **33**:267–293. 1919.

[19] KIDD, FRANKLIN, Laboratory experiments on the sprouting of potatoes in various gas mixtures. New Phytol. **18**:248–252. 1919.

even injuring growth as do some other anesthetics with the potato. In work of this kind one should be very sure that the gases used carry no other injurious gases.—WM. CROCKER.

Modification of unit characters.—An epoch in the perennial controversy between "mutationists" and "selectionists" is marked by CASTLE'S[20] shift from the latter to the former school. This investigator has previously held a leading place among "selectionists," with his modification by selection of the hooded character of rats. His change in point of view has been effected mainly by some of his own results. The cross between his plus race (+3.73) of hooded rats and a wild race brought a reduction in the grade of the hooded character as it appeared in the extracted hooded F_2 young. Repeated recrossing of these extracted individuals with the wild race finally resulted in extracted hooded rats of the grade +3.04. CASTLE concluded that the hooded character had been modified to this degree by its successive contacts with the germ plasm of the wild race. More recently he crossed his minus race (−2.63) with the same wild race. Repeated F_2 extractions showed successively the grades −0.38, +1.01, +2.55, and one family reached +3.05. These results indicate clearly that the hooded character in the plus and minus races are identical, only the multiple modifying factors differing. Repeated crosses with the wild race eventually produced hooded individuals whose quota of modifying factors approximated that of the wild race, evidently represented by a grade of +3.04 or +3.05. Consistent with the idea of a single unit for the hooded character and multiple modifying factors, the successive hooded populations that were extracted showed a decreasing degree of variability.—M. C. COULTER.

Rainfall efficiency.—The well known fact that plant foliage intercepts a considerable amount of the rainfall has been emphasized recently by MCLEAN[21] and others. A decidedly valuable contribution to the subject is represented by the extensive data of HORTON,[22] who has shown that the average observed interception during the summer of 1918 was 40 per cent of the precipitation. This loss ranges from 25 per cent for rains of long duration to 100 per cent for light showers, and seems to be nearly the same for most broad-leaved trees during the summer. These interception losses are greater for needle-leaved trees than for broad-leaved ones. Although the data are still insufficient to make an accurate comparison of the losses occurring at different seasons of the year, it is clear that since light showers are most frequent during the summer season the losses will be greatest during such a period, or in other

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[20] CASTLE, W. E., Piebald rats and the theory of genes. Proc. Nat. Acad. Sci. 5:126–130. *fig. 1*. 1919.

[21] MCLEAN, R. C., Studies in the ecology of tropical rain-forest. Jour. Ecol. 7:121–172. 1919.

[22] HORTON, R. E., Rainfall interception. Mo. Weather Rev. 47:603–623. *figs. 17*. 1919.

words, the efficiency of precipitation is least during the period of greatest need. The interception by full grown field crops is comparable in value with that from trees.

Selections from previously collected data and a short bibliography add their value to HORTON's report.—GEO. D. FULLER.

Hawaiian Lobelioideae.—ROCK[23] has published an elaborately printed and profusely illustrated monograph of the Hawaiian Lobelioideae. The very numerous photographic reproductions make the monograph almost equivalent to an herbarium set of the material. The tribe represents the family Lobeliaceae as ordinarily presented, and in the Hawaiian Islands includes 7 genera, 6 of which are endemic. The author has been studying this group for nearly 10 years, and has increased the 58 species of HILLEBRAND'S *Flora* to 104, all peculiar to the Islands; and in his opinion many more species will be brought to light, especially in the genus *Cyanea*, which in the monograph includes 52 species. The only genus of world-wide distribution is *Lobelia*, which is credited with 11 species in the Islands, 4 of which are new. The first part of the monograph contains a general discussion of structure, habit, and distribution. —J. M. C.

Evolution of cotyledony.—BUCHHOLZ[24] has investigated the ontogeny of the cotyledons in a number of living conifers, and has reached some important conclusions. He finds in certain conifers a considerable number of primordia, which may develop a corresponding number of cotyledons, or fusions may occur, thus reducing the number. In no case was there any evidence of increasing the number of cotyledons by splitting. The fusions resulting in a reduced number of cotyledons in some cases resulted also in cotyledonary tubes. The inference is that polycotyledony is primitive; that dicotyledony was attained by a general fusion of many cotyledons into two groups or by a bilabiate development of the cotyledonary tube; and that monocotyledony is the result of a cotyledonary tube becoming unilabiate. This evolutionary sequence seems to be borne out by all the facts at hand, and relates the different forms of cotyledony in a natural way.—J.M.C.

Proceedings of the Indiana Academy.—The volume of Proceedings of the Indiana Academy of Science for 1918 has just been distributed, including 327 pages and numerous illustrations. Among the botanical contributions published are the following: The barberry and its relation to the stem rust of wheat in Indiana, F. J. PIPAL; A method of teaching diffusion and osmosis

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[23] ROCK, JOSEPH F., A monographic study of the Hawaiian species of the tribe Lobelioideae, family Campanulaceae. Publ. Bernice Pauahi Bishop Museum. 4to pp. xvi+395. *pls. 217*. 1919.

[24] BUCHHOLZ, JOHN T., Studies concerning the evolutionary status of polycotyledony. Amer. Jour. Bot. **6**:106–119. *figs.* 25. 1919.

in connection with biological work, PAUL WEATHERWAX; Bacteria in frozen soil, H. A. NOYES; Some abnormalities in plant structure, M. S. MARKLE; Plants of Boone County, Kentucky, JAMES C. NELSON; Plants new to Indiana. VIII, CHARLES C. DEAM; Analyses of 100 soils in Allen County, Indiana, R. H. CARR and V. R. PHARES; The relation of nitrogen, phosphorus, and organic matter to corn yield in Elkhart County, Indiana, R. H. CARR and LEROY HOFFMAN; Soil survey of Cass County, Indiana, COLONZO C. BEALS; Ascomycetes new to the flora of Indiana, BRUCE FINK and SYLVIA C. FUSON; The dormant period of timothy seed after harvesting, M. L. FISHER.—J. M. C.

Douglas firs.—HENRY[25] and FLOOD have described three American and four Asiatic species of *Pseudotsuga*, separating the Pacific Coast trees from those found in the Rocky Mountains. Aside from some minor differences in leaf and cone structure, the authors believe that the Rocky Mountain form, *P. glauca*, shows more xerophytic structures and is much more resistant to injury by frost and drought. The differences in the behavior of the two forms under silvicultural conditions in Great Britain seems to afford a much better basis for considering the eastern form a separate species. Of the Asiatic species, one is native to Japan, one to Formosa, and two are native to Yunnan, China. All are found in restricted areas and are to be regarded as so rare as to be of little economic importance.—GEO. D. FULLER.

Evaporation and vapor pressure deficit.—It has been shown by JOHNSTON[26] that it is possible, by using vapor pressure deficit and wind velocity data, the former being derived from hygrometer and thermometer readings, to calculate the "potential evaporation" or evaporating power of the air in a manner that will show a very close agreement with the records from the porous cup atmometer. In this way considerable data collected by the Weather Bureau may be translated into terms that are significant and valuable for the ecologist.—GEO. D. FULLER.

Plantago in Hawaii.—ROCK[27] has monographed the two endemic species of *Plantago* occurring in the Hawaiian Islands. One of them, *P. princeps*, is a branching shrub, and its variability is indicated by the fact that 8 varieties are recognized. The other species, *P. pachyphylla*, includes 7 varieties, among which there is a new one (var. *anomala*) which combines the characteristic capsule and venation of *P. pachyphylla* with the seeds and arborescent branching habit of *P. princeps*.—J. M. C.

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[25] HENRY, A., and FLOOD, MARGARET G., The Douglas firs: a botanical and silvicultural description of the various species of *Pseudotsuga*. Proc. Roy. Irish Acad. **35**: sect. B. 67–92. *pls. 12–14.* 1920.

[26] JOHNSTON, E. S., Evaporation compared with vapor pressure deficit and wind velocity. Mo. Weather Rev. **47**:30–33. *figs. 2.* 1919.

[27] ROCK, J.F., The genus *Plantago* in Hawaii. Amer. Jour. Bot. **7**:195–210. *pl. 13.* 1920.

VOLUME LXX NUMBER 5

THE

BOTANICAL GAZETTE

NOVEMBER 1920

NORTH AMERICAN SPECIES OF TARAXACUM[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 272

EARL EDWARD SHERFF

(WITH PLATES XXXI–XXXIII)

For many years our knowledge of the American species of *Taraxacum* has been in a very imperfect and chaotic state. The perusal of the more prominent manuals and floras issued in the United States during the past few decades shows a surprising confusion of forms and multiplicity of specific names. This confusion is easily accounted for by the fact that most of the *Taraxacum* forms tend strongly to intergrade, so much so that many botanists in the past have despaired of their specific segregation. Thus TORREY and GRAY (Fl. N. Amer. **2**:494. 1843), after describing *Taraxacum dens-leonis* (=*T. vulgare*), wrote as an introduction to their four additional species: "The following species (the characters of which we copy from chiefly DE CANDOLLE, who keeps them distinct), as well as nearly all the genuine *Taraxaca*, are not improbably

[1] Including the West Indies, but not Greenland. The large number of new species recently proposed for Greenland by DAHLSTEDT have made it inadvisable to include the Greenland plants until a more abundant supply of Greenland material can be obtained for detailed study. So far, however, I have examined no plants from Greenland that were not clearly referable either to those species included in this treatment or to *Taraxacum nivale* Lange, a species close to *T. lyratum* but differing in having the achenes glabrous or nearly so. From a study of DAHLSTEDT'S work (Archiv f. Botanik 4^8:1–41. 1905; *ibid.*, 5^9:1–44. 1906) and those of his determinations accessible to me, it appears that his "new species" are mostly synonymous with *Taraxacum lyratum*, *T. nivale*, and *T. ceratophorum*.

correctly viewed by FRIES, KOCH, and other excellent botanists, as mere varieties of this, the *Common Dandelion*."[2] At a later date we find that GRAY himself (Synopt. Fl. N. Amer. 1²:440. 1884) had come to regard all the continental North American forms as representing varieties of but one species, which he stated to be a "very polymorphous species."

In 1907 there appeared the classic monograph of *Taraxacum* by HANDEL-MAZZETTI. This author, evidently impressed with the desultory treatment which the genus usually had been accorded in previous studies, quoted the relevant words of REICHENBACH (Fl. Germ. Excurs. 270. 1830–1832), which are here translated: "A genus seriously forsaken heretofore, because of the negligence of writers. A positively tedious comparison of leaves, without the remaining points having been carefully investigated and clearly set forth, renders the amateurs fully as confused as are the botanists themselves. Moreover, the fruits especially must be observed, and these only in their mature state."

In HANDEL-MAZZETTI'S work there was given an admirable presentation of the various species of the genus. Even this valuable monograph, however, was rather inadequate for a critical opinion of the North American species, since there were a number of the more recently proposed species of which he obviously had not seen authentic specimens. In certain other cases his examination of American specimens was too limited, and I fail to find even the slightest mention of some of the species proposed by American authors previous to 1907. What seems worst of all, however, is that the many valid results of his research have been passed by almost unnoticed until the present day in this country. Taxonomic literature relating to *Taraxacum* in America is still weighted with inaccuracies that ought to be corrected.

The study presented herewith was undertaken in 1918. From the beginning the main purpose has been to correlate much of the material in American herbaria with HANDEL-MAZZETTI'S treatment, to corroborate his results where possible, to correct or improve and to augment where there was need, and then to present

[2] For reference to the works of FRIES, KOCH, and VOITH, touching this point, see DC. Prodr. 7:145 (footnote). 1838.

the conclusions for their easier accessibility to American students. The full generic description given by HANDEL-MAZZETTI is omitted, nor has it been attempted to repeat his extensive lists of synonyms in full. Only such synonyms are given as seem vital or as were misplaced or overlooked by him. For the sake of comparisons, however, his excellent specific descriptions have been followed closely; in the main, only such alterations have been made as considerations of brevity or accuracy would dictate.

Since the completion of my research there has appeared the recent article by STORK (Bull. Torr. Bot. Club **47**: 199–210. *pls. 6, 7.* 1920). It contains interesting data concerning sexuality, variation, and cytological aspects of certain American species, and cites several references that must be omitted here.

As may be noted in the following pages, I have found no occasion for proposing a single new species. In fact the literature of the genus has suffered seriously in the past from the persistent and repeated proposal of ill-advised and scantily considered specific names, many of them founded upon freakish or even immature material. Rather have I been compelled to reduce three species retained by HANDEL-MAZZETTI (*T. phymatocarpum*, *T. mexicanum*, and *T. lapponicum*) to synonomy, thus giving a total of only five species in our range.

Most of the work was prosecuted in the Herbarium of Field Museum, where I was afforded the fullest measure of freedom and courtesy through the kindness of the Curator, Dr. CHARLES F. MILLSPAUGH. The entire collections of *Taraxacum* in the United States National Herbarium, especially rich in specimens from Alaska and the Arctic regions, were loaned through the generosity of the Associate Curator, Mr. WILLIAM R. MAXON. All of the highly important materials in the Herbarium of the Canadian Geological Survey were loaned by Professor JAMES M. MACOUN, late of that Herbarium. Among these were a vast number of valuable specimens from western Canada and the Arctic regions. All of the nearly 800 specimens in the Herbarium of Boissier and the Herbarium of the Institut de Botanique, Geneva, were loaned by Dr. R. CHODAT. These, while mostly from Europe, Asia, and elsewhere than from North America, were of the greatest value in

promoting a better evaluation of the North American species. On two occasions I was permitted by Dr. JULIUS NIEUWLAND to study freely the specimens, several of them types, in the Greene Herbarium at the University of Notre Dame. To all of these botanists I here express my great indebtedness and gratitude. Others to whom also I am grateful for assistance rendered are: Dr. N. L. BRITTON, Director of the New York Botanical Garden; Dr. J. M. GREENMAN, Curator of the Herbarium, Missouri Botanical Garden; Dr. AVEN NELSON, President, University of Wyoming; Professor W. W. ROWLEE, Cornell University; Professor D. B. SWINGLE, Botanist and Bacteriologist, Montana Agricultural Experiment Station.

Abbreviations used for Herbaria: Hb. Boiss., Herb. Boissier; Hb. Can., Herb. Canadian Geological Survey; Hb. Chi., Herb. University of Chicago; Hb. Field, Herb. Field Museum of Natural History; Hb. N.Y., Herb. New York Botanical Garden; Hb. U.S., United States National Hebarium.

Clavis specierum

Achaenia matura rubescentia
- Folia plerumque gracilia, profunde laciniato-pinnatifida; involucri foliola exteriora plerumque patentia vel etiam recurvata . . . . . 5. *T. laevigatum*
- Folia crassiora, minus profunde pinnatifida vel etiam integra; involucri foliola exteriora plerumque adpressa . . . . . 3. *T. eriophorum*

Achaenia matura non (nisi interdum versus apicem in num. 1) rubescentia
- Achaenia matura nigrescentia (vel interdum versus apicem rubescentia) 1. *T. lyratum*
- Achaenia matura aliusmodi
  - Involucri foliola plerumque adpressa et corniculis instructa 2. *T. ceratophorum*
  - Involucri foliola ecorniculata vel raro corniculis minimis instructa, exteriora patentia vel reflexa . . . . . 4. *T. vulgare*

1. TARAXACUM LYRATUM (Led.) DC., Prodr. **7**:148. 1838; *Leontodon lyratus* Ledebour, Icon. Pl. Fl. Ross. ill. **5**:27. *pl. 497*. 1834; Fl. Altaica **4**:152. 1833; *T. phymatocarpum* Vahl, Fl. Danica **13** (fasc. 39):6. *pl. 2298*. 1840; Watson, U.S. Geol. Explor. 40th Parallel 207. 1871; *T. laevigatum* Gray, Proc. Acad. Phil. 1863, p. 70 (non Willd.); *T. officinale* var. *scopulorum* Gray, Synopt. Fl. N. Amer. **1**^{2}:440. 1884; *T. Taraxacum* var. *scopulorum* Heller,

Cat. N. Amer. *pl. 8.* 1898; *T. scopulorum* Rydb., Mem. N.Y. Bot. Gard. **1**:455. 1900; *T. rupestre* Greene, Pittonia **4**:229. (Jan.) 1901; *T. alaskanum* Rydb., Bull. Torr. Bot. Club **28**:512. (Sept.) 1901; *T. hyperforeum* Dahlst., Videsk.-Selsk. Christ. Math.-Naturv. Kl. 1909: no. 8. p. 26. 1910; *T. eurylepium* Dahlst., *loc. cit.* 72; *T. fasciculatum* Nels., Bot. Gaz. **56**:71. 1913 (ex descript. et altitudine); *Leontodon rupestre* Rydb., Fl. Rocky Mts., 1035. 1917; *L. scopulorum* Rydb., *loc. cit.*—Pl. XXXI.

Herba valde variabilis, pusilla (interdum minima), 2–15 (–25) cm. alta. Radix tenuiuscula vel crassa, simplex vel multiceps, collo subsquamato vel foliorum vetustorum fragmentis persistentibus squamato, glabro vel sparse piloso vel sparse longo lanuginoso. Folia suberecta vel patentia, tenuia, glabra, lanceolata vel spathulata, 3–15 mm. lata, infra saepe longe attenuata, nunc integra, nunc denticulata vel sinuato-dentata, nunc regulariter incisa, lobis triangularibus vel lanceolatis, acutis vel obtusis, integris vel subdenticulatis, subrecurvis vel patentibus. Scapi plerumque pauci vel singuli, tenues, glabri vel iuveniles sub capitulo densiuscule villosi, florendi tempore foliis breviores vel multo longiores. Capitula minima (circum 7–12 mm. longa nec latiora) vel maiora (circum 12–18 mm. longa et paulo latiora). Involucri foliola pauca, atroviridiora vel nigricantia, ecorniculata vel corniculis parvis saepe instructa; exterioris seriei foliola adpressa vel laxe adcumbentia, interiorum angustiorum longitudinis 0.4–0.5 attingentia, infima vix breviora, omnia latius angustiusve ovata (latitudine 0.5–1.5 longiora), margine decolorato variabili vel nullo. Flores pauci vel numerosi, sulphurei vel flavi, involucro 1–4 mm. longiores. Achaenia maiuscula, ad 5 mm. longa, atra, fere nigra (versus apicem saepe subrubida), iuniora brunnea, tota rugosa, tuberculis largis, supra longioribus, crassis tenuioribusve obsita, abrupte in cuspidem crasse cylindricam, sulcatam, brevem vel brevissimam, totius fructus septimam partem aequantem vel quintam vix superantem contracta. Rostrum strictum, achaenio subaequilongum. Pappus illo paulo longior, albus.

Distribution.—Greenland, Arctic America, and northeastern Asia, southward at alpine heights along the mountains of western Canada and the western United States to Arizona.

SPECIMENS EXAMINED.—Alaska (and neighboring islands): Hall Island, July 14, 1899, *Brewer* and *Coe* 427 (Hb. Greene 30831); Point Gustavus, Glacier Bay, June 10–12, 1899, *Coville* and *Kearney* 732 (Hb. U.S. 376695); Haenke Island, Disenchantment Bay, June 22, 1899, *iidem* 1097 (Hb. U.S. 376702); St. Matthew Island, Bering Sea, July 15, 1899, *iidem* 2164 (Hb. U.S. 376718); Bennet, July 31, 1907, *Henry C. Cowles* 1005 (Hb. Field 419544); Clifton Point, Dolphin-Union Straits (lat. 69° 13′N.), in 1916, Rev. *H. Girling* (Hb. Can. 99071); Herald Isl., Arctic Ocean, in 1881, *Capt. C. L. Hooper* (Hb. U.S. 424060); Wollaston Land (lat. 69–70° N., long. 115° W.), July 1915, *D. Jenness* 415 (Hb. Can. 98711); Camden Bay (lat. 70° N., long. 145° W.), Collinson Point, July 17, 1914, *Frits Johansen* 115 (Hb. Can. no 98716 in Hb. Field 483378); Bernard Harbor (lat. 68° 47′ N., long. 114° 46′ W.), August 1914, *idem* 276 (Hb. Can. 98715); Cape Bathurst (lat. 70° 35′ N., long. 128° 6′ W.), July 1916, *idem* 508 (Hb. Can. 98713); Popof Isl., Shumagin Isls., July 14, 1899, *Trevor Kincaid* (Hb. U.S. 376796); Muir Glacier, July 8–19, 1899, *idem* (Hb. U.S. 376795, peculiar form with foliage of the *T. alaskanum* form and with achenes brown as in *T. ceratophorum*); Point Barrow (lat. 71° N.), steep side bank facing the ocean, July 23, 1898, *E. A. McIlhenny* (Hb. N.Y.; Hb. Can. 26283; type and cotype of *T. alaskanum* Rydb.); Point Barrow, in 1883, *Dr. John Murdoch* (Hb. U.S. 424062, 424063, and 424064, topotypes of *T. alaskanum* Rydb.); Kadiak Isl, vicinity of Karluk, July 5, 1903, *Cloudsley Rutter* 197 (Hb. U.S. 420615, the form matching LEDEBOUR's type illustration very closely); vicinity of Port Clarence, Teller Reindeer Station, tundra banks near beach July 20, 1901, *F. A. Walpole* 1492 (Hb. U.S. 378605), August 9, 1901, *idem* 1791 (Hb. U.S. 378905), and September 4, 1901, *idem* 1987 and 1988 (Hb. U.S. 379107 and 379108 respectively); vicinity of Port Clarence, banks along streams, flat west end of Tasuk Lagoon, August 22, 1901, *idem* 1895 (Hb. U.S. 379011); Arakamtchetchene Isl., Bering Staits, *C. Wright* in 1853–1856 (Hb. U.S. 424059).

British Columbia: West Summit of S. Kootenay Pass, mountain slopes, July 26, 1883, *Dawson* (Hb. Can. 15115); Chilliwhack River, alt. 6000 ft., rocky slopes, August 29, 1901, *J. M. Macoun* (Hb. Can. 26811); Hazelton, Skeena River, mountains, alt. 4500 ft., July 13, 1917, *idem* (Hb. Can. 98703; Hb. Field 483397); second summit west of Skagit River, July 24, 1905, *idem* (Hb. Can. 77001); Cascade Range, near head of McGillivray Creek, alt. 6500 ft., August 12, 1916, *idem* (Hb. Can. 98701; Hb. Field 483396); Mt. Queest, alt. 6000 ft., crevices of rocks, July 25, 1889, *idem* (Hb. Can. 15111; type of *T. rupestre* Greene); Kicking Horse Lake, alt. 8000 ft., alpine slopes, August 14, 1890, *idem* (Hb. Can. 11114; Hb. U.S. 219543); Kicking Horse Lake, damp open thickets, July 28, 1885, *John Macoun* (Hb. Can., 15110); Avalanche Mt., Selkirk Mts., alt. 8000 ft., August 4, 1890, *idem* (Hb. Can., *sine num.*); Revelstoke, alt. 1600 ft., July 26, 1905, *C. H. Shaw* 1008 (Hb. U.S. 621912); Tete Jaune Cache, headwaters of Fraser River, mountain summits, August 31, 1898, *W. Spreadborough* (Hb. Can. 19743).

Alberta: Below and at Ottertail Pass (Rocky Mt. Nat. Park), alt. 6900 ft., August 10, 1904, *John Macoun* (Hb. Can. 65620; Hb. Field 222849); Crow Nest Pass, mountain slopes, alt. 7000 ft., August 6, 1897, *idem* (Hb. Can. 23109); Fitzhugh Mt., Jasper Park, alpine summits, alt. 7000 ft., August 8, 1917, *J. M. Macoun* (Hb. Can. 98682, 98683 and 98684; Hb. Field 483384); Shovel Pass, Jasper Park, high slopes and summits, alt. 7000 ft., August 10, 1918, *idem* (Hb. Can. 98679; Hb. Field 483381); Shovel Pass, Jasper Park, among rocks at foot of cliff, alt. 6000 ft., August 17, 1918, *idem* (Hb. Can. 98680; Hb. Field 483382); Goat Mt., Jasper Park, above tree limit, alt. 7000 ft., July 18, 1918, *idem* (Hb. Can. 98681; Hb. Field 483383); Mt. Edith Cavell, Jasper Park, damp flat, alt. 6000 ft., *idem* (Hb. Can. 98690; Hb. Field 483389).

Montana: Old Hollowtop, near Pony, July 9, 1897, alt. 9000 ft., *Rydberg* and *Bessey* 5294 (Hb. U.S. 361402); above Stanton Lake, alt. 7000–7500 ft., August 1–7, 1894, *R. S. Williams* 1073 (Hb. Greene 48454; Hb. U.S. 288541).

Wyoming: Big Horn Mountains, alt. 10000 ft., July 17, 1890, anonymous (Hb. Greene 48456); without locality, *F. Tweedy* 745 pro parte (Hb. U.S. 41953).

Colorado: Mt. Hesperus, alt. 11000 ft., July 2, 1898, *Baker*, *Earle*, and *Tracy* 293 (Hb. Field 76097; Hb. Chi. 356356; Hb. U.S. 337212); Saguache (Sawatch) Range, alt. 12000 ft., August 1880, *T. S. Brandegee* (Hb. Field 204736); Uncompahgre River, mountain slopes, alt. 12000–13000 ft., August 1893, *C. A. Purpus* 719 (Hb. Chi. 357798).

Utah: La Sal Mts., alt. 3000–3300 m., July 7, 1911, *Rydberg* and *Garrett* 8720 (Hb. Can. 85360; Hb. U.S. 765075); Uintah Mts., above Bear River, alt. 12000 ft., August 1869, *Sereno Watson* 724 (Hb. U.S. 41943).

Nevada: Rocky Mountains, July 20, 1896, *Edward L. Greene* (Hb. Greene 48455).

Arizona: San Francisco Mts., August 27, 1889, *F. H. Knowlton* 142 (Hb. U.S. 41949).

LEDEBOUR founded his species upon Asiatic material with immature fruit, collected in stony places upon an alpine summit along the Tschuja River opposite the mouth of the Tschegan River. Several of the specimens from Alaska (for example, *Coville* and *Kearney* 1097, *Rutter* 197) match his description and plate, also specimens of the type collection (legit BUNGE, Hb. Boiss.) very closely. Many other Alaskan specimens fail to have the lateral laciniae of the leaves ovate, as described by LEDEBOUR, but acute instead. Here must be placed *T. alaskanum* Rydb., of which I have examined the type sheet, also the cotype in the Herbarium of the Canadian Geological Survey. Proceeding south from Alaska, forms may be found coming from the high alpine

altitudes of Colorado, Utah, etc., that in some cases look even specifically distinct. Such plants (for example, *Baker*, *Earle*, and *Tracy* 293, *Tweedy* 745 pro parte) are commonly dwarfed, 2–3.5 cm. high, and their diminutive involucres measure sometimes as low as 4–6 mm. in width at base during anthesis. It is these plants that GRAY named *T. laevigatum*, and later *T. officinale* var. *scopulorum*. A study of numerous other specimens, however, especially from Montana, Alberta, and British Columbia, reveals all possible intergradations between the two extremes of foliage and involucre. One of these forms is the *T. rupestre* Greene, of which I have studied the type and all the other material cited by GREENE.

Recently RYDBERG (*loc. cit.*) has created the name *Leontodon scopulorum* for the dwarf alpine forms of the Rocky Mountains, but, as both HANDEL-MAZZETTI and I have finally concluded, this dwarf form is entirely inseparable from *T. lyratum*. Also, for those who discard the name *Taraxacum* but persist in employing the name *Leontodon*, the name *Leontodon lyratus*, as it was originally published by LEDEBOUR, should suffice.

HANDEL-MAZZETTI had seen no mature fruit of the materials regarded by him as *T. lyratum;* but a duplicate (*Jas. M. Macoun*, Mts. at Kicking Horse Lake, British Columbia, Hb. U.S. 219543) of one of the specimens cited by himself (and seen by him at the University of Vienna) has several mature achenes, which are black. Numerous other Canadian specimens examined have likewise black or blackish achenes, but in certain cases these achenes are slightly reddish near the top. At times sheets of material are observed on which the specimens have variously few, many, or all of their leaves spatulate or lanceolate, with margins merely dentate or even subentire. Typical examples of this kind are: *Knowlton* 142, Arizona (Hb. U.S. 41949); *Macoun*, British Columbia (Hb. Can. 98701; Hb. Field 483396); *Coville* and *Kearney* 1097, Alaska (Hb. U.S. 376702); *Walpole* 1791, 1895, and 1987, Alaska (Hb. U.S. 378905, 379011 and 379107 respectively). These specimens are extremely important, for some of them match the specimens of *T. phymatocarpum* from Greenland so minutely that all attempts at separation are fruitless. HANDEL-MAZZETTI (*loc. cit. pl.* 7) presents a distributional map in which he shows *T. lyratum* ran-

ging from southern Colorado northwestward through British Columbia, Alaska, and barely touching Asia.[3] For *T. phymatocarpum* he gives a more northern range, extending from Greenland westward through Alaska and slightly into Asia. My own study, however, leaves me entirely unable to maintain such a separation. To do so would necessitate in many instances actually taking materials on the same sheet, collected at the same time and place, and known to be even racially the same, and dividing them arbitrarily between the two "species," a manifestly absurd and indefensible procedure. In this connection it is interesting to note that, years ago, SERENO WATSON determined a specimen collected by himself in Utah (*Watson* 724, Hb. U.S. 41943) as *T. phymatocarpum* Vahl. He stated expressly on the label that his determination was "fide speciminis in Groen. a Rink lecti." Thus WATSON likewise was convinced of the identity of the Utah material with that of Greenland.[4]

T. fasciculatum Nels. was described from flowering specimens collected by *Alfred A. Griffin* (no. 111) from Wagon Wheel Gap, Blue Park, Colorado, alt. 11000 ft., July 21, 1912. NELSON has been unable to locate the type specimen for me, but the description ("few-several oblanceolate or oblong obtusish merely dentate or denticulate subsessile or short-petioled glabrous leaves 4–7 cm. long"), together with the high altitude recorded, indicates clearly that the plant was *T. lyratum* of the form that, from Greenland, has heretofore been termed *T. phymatocarpum*.

Occasionally a form of *T. lyratum* is found closely simulating the form of *T. ceratophorum* which GREENE described as *T. mutilum*, and differing clearly from "*T. mutilum*" only in having black achenes (for example, *Walpole* 1791 and 1987, Alaska, Hb. U.S. 378905 and 379107 respectively; *Dr. Murdoch*, Alaska, Hb. U.S. 424062 and 424064). Its foliage is long linear or linear-lanceolate, remotely and very sharply toothed. This form matches very closely the type illustrations of *T. hyperboreum* Dahlst., from Gjöa

[3] The type of *T. lyratum*, however, was collected in the interior of Asia!

[4] Elsewhere (U.S. Geol. Explor. Fortieth Parallel 207. 1871) WATSON said: "The present specimen, a single one only, is rather larger than those from Greenland, but is plainly the same plant."

Harbor, lat. 68° 37′ 38″ N., long. 96° 23′ 40″ W., and *T. eurylepium* Dahlst., from Herschell Island (cf. pl. XXXI fig. *c*). DAHLSTEDT had seen no achenes for either of his two proposed species, but a study of *Walpole* 1987 reveals the black achenes, as in typical *T. lyratum*. Numerous variations in foliage and involucre connect the form clearly with true *T. lyratum*, and make it impossible to draw any specific distinctions.

2. TARAXACUM CERATOPHORUM (Led.) DC. Prodr. **7**:146. 1838; *Leontodon ceratophorus* Ledebour, Icon. Pl. Fl. Ross. **1**:9. *pl. 34.* 1829; Fl. Altaica **4**:149. 1833; *T. montanum* Nutt. (non Mey. et DC.), Trans. Amer. Phil. Soc. n.s. **7**:430. 1841; WOOTON and STANDLEY, Contrib. U.S. Nat. Herb. **19**:627. 1915; *T. lividum* Heller, Bull. Torr. Bot. Club **24**:480. 1897 (exclud. synon. Waldst. et Kit.); *T. Chamissonis* Greene, Pittonia **4**:228. 1901; *T. lacerum* Greene, *loc. cit.* 230; *T. dumetorum* Greene, *loc. cit.* 230; *T. mutilum*, Greene, *loc. cit.* 239; *T. leiospermum* Rydb., Bull. Torr. Bot. Club **32**:137. 1905; *T. oblanceolatum* Nels. ex Rydb., Fl. Colorado 410. 1906 (ex synon. *T. dumetorum* Greene);[5] *T. lapponicum* Handel-Mazzetti, Monogr. Taraxacum 73. 1907 (saltem quantum ad plantas americanas, forsan non Kihlm.); *Leontodon dumetorum* Rydb., Fl. Rocky Mts. 1035. 1917; *L. leiospermum* Rydb., *loc. cit.*; *L. monticola* Rydb., *loc. cit.*—Pl. XXXII.

Herba valde polymorpha, plerumque robustior, 7–25 (–35) cm. alta. Radix crassiuscula, nigrescenti-corticata, collo haud vel vix squamato, glabro vel sparsissime lanato. Folia laxe procumbentia, adscendentia vel erecta, herbacea, viridia vel pallida, glabra vel infra sparsissime pilosa, lanceolata vel oblanceolata, 1–6 (–9) cm. lata, infra saepe longe attenuata, ad apicem acuta vel obtusa, leviter sinuato-dentata vel variis modis runcinato-incisa, raro integra vel tenuissime dissecta, lobis acutis, latius angustiusve triangularibus, integris vel dentatis, acutis, lobo terminali plerumque maiore. Scapi singuli vel numerosi, suberecti, florendi tempore foliis plus minusve aequilongi, denique elongati, iuveniles plus minusve lanato-pilosi. Capitula magna, 1.5–2.5 cm. alta et 2–5 cm. lata. Involucrum griseo-viride vel nigrescens, interdum pruinosum. Involucri foliola corniculis plus minusve

[5] AVEN NELSON 8236, distributed by NELSON as *T. oblanceolatum*, is likewise referable to *T. ceratophorum*.

atratis et apicem dilatatum saepe superantibus fere semper instructa, exteriora adpressa vel patentia, late ovata vel lanceolata, interiorum longitudinis $\frac{1}{6}$–$\frac{3}{5}$ (vel raro totum) aequantia, plerumque 5–15 mm. longa, margine decolorato interdum nullo sed saepius praesente et bene distincto. Flores numerosi, magni, foliolis 5–10 mm. longiores, flavi vel sulphurei. Achaenia 4–5 mm. longa, straminea vel brunnea vel griseo-brunnea, supra tuberculis angustis mediocris longitudinis dense obsita et saepe tota rugulosa, in cuspidem crassam vel angustam, brevem vel tertiae parti totius fructus aequantem cuneate attenuata. Rostrum tenue, achaenio paulo vel multo longius. Pappus albus, 5–8 mm. longus.

DISTRIBUTION.—Labrador and Alaska southward at higher altitudes to New Hampshire, Massachusetts, Montana, New Mexico, and California; in the entire Arctic region, the mountains of Central Asia, and even "in the Caucasus and in the Alps of Switzerland (a single locality)."

SPECIMENS EXAMINED.[6]—Labrador (Peninsula): Northern Labrador along the Ungava River, August 20, 1896, *Spreadborough* (Hb. Can. 14395); Ungava, *Lucien M. Turner* 613 (Hb. U.S. 222756).

Quebec: Banks of the Grand River, Gaspe County, June 30–July 3, 1904, *M. L. Fernald* (Hb. Field 465065; Hb. U.S. 605794); Rimouski County, July 4, 1907, *Fernald* and *Collins* 1210 (Hb. Can. 86493).

Keewatin: West Coast of Hudson Bay, lat. 56° N., sandy grounds, August, 1886, *James M. Macoun* (Hb. Can. 15112); Churchill, Hudson Bay, lat. 58° 50′ N., July 26, 1910, *idem* (Hb. Cornell Univ.; Hb. Can. 79286; Hb. Field 295238; important as matching exactly the form described by GREENE for his *T. mutilum*).

Manitoba: Birtle, vicinity of, along G.T. Pacif. R.R., June 26, 1906, *Macoun* and *Herriot* (Hb. Can. 77046); Forest, six miles east of, along G.T. Pacif. R.R., June 19, 1906, *iidem* (Hb. Can. 77047); Oak River, along G.T. Pacif. R.R., June 21, 1906, *iidem* (Hb. Can. 77048).

Mackenzie: Cape Barrow (south coast of Coronation Gulf), August 9, 1915, *Cox* and *O'Neil* 451 (Hb. Can. 98712; Hb. Field 483375); Fort Resolution, July 14, 1903, *Edward A. Preble* 210 (Hb. U.S. 421694).

[6] Many specimens are omitted for lack of space. As representing the extreme form with bracts ecorniculate (*T. lapponicum*), there may be added the following examples: Alberta: Near Old Man's River, damp grassy places, August 4, 1883, *Dawson* (Hb. Can. 15124). Wyoming: Northwestern part of state, August 9, 1893, *J. N. Rose* 679 (Hb. U.S. 41951). Utah: Tate Mine, near Marysvale, alt. 9000 ft., August 22, 1894, *Marcus E. Jones* 5853 (Hb. U.S. 233114); Gold Basin, La Sal Mountains, alt. 3000–3300 m., July 11, 1911, *Rydberg* and *Garrett* 8836 (Hb. U.S. 765101). California: Bear Valley, San Bernardino Mountains, in meadows, August, 1882, *S.B.* and *W. F. Parish* 1461 (Hb. Field 208755; Hb. U.S. 783095); Bear Valley, San Bernardino Mts., alt. 6500 ft., June 18, 1894, *S. B. Parish* 3131 (Hb. U.S. 214378).

Saskatchewan: Moose Jaw, open ground by the creek, June 20, 1896, *John Macoun* (Hb. Can. 12737); Moose Jaw, vicinity of, July 13, 1895, *idem* (Hb. Can. 11713); Prince Albert, camp thickets, June 29, 1896, *idem* (Hb. Can. 12283); Wood Mountain Post, thickets, June 11, 1895, *idem* (Hb. Can. 11712); Cypress Hills, thickets, June 24, 1894, *idem* (Hb. Can. 5087; labeled in GREENE'S handwriting as being "part of type" of his *T. dumetorum*); Cypress Hills, springy places, June 2, 1884, *J. M. Macoun* (Hb. Can. 15131).

Assiniboia: Medicine Hat, June 8, 1894, *John Macoun* (Hb. U.S. 232067).

Montana: Bridger Mountains, alt. 7000 ft., June 14, 1897, *Rydberg* and *Bessey* 5295 (Hb. Can. 40007; Hb. Field 81947; a form having atypic foliage, possibly a hybrid); Midvale, plains, June 17, 1903, *L. M. Umbach* 75 (Hb. Field 191120; Hb. U.S. 541438); Highwood Mts., June 19, 1888, *R. S. Williams* 434 (Hb. U.S. 288542).

Wyoming: Yellowstone National Park, July 13, 1902, *Edgar A. Mearns* 1779 (Hb. U.S. 486830); Pacific Creek, 65 miles north of Point of Rocks, June 22, 1901, *Merrill* and *Wilcox* 575 (Hb. U.S. 580684).

Colorado: Ruxton Dell, alt. 2900 m., July 17, 1903, *F. E.* and *E. S. Clements* "363.1" (Hb. U.S. 580390); Camp Creek, Larimer County, semi-meadow land, July 6, 1903, *Leslie N. Goodding* 1462 (Hb. U.S. 581396); without locality (lat. 39–41° N.), in 1862 *Hall* and *Harbour* 357 (Hb. Field 17783 314685; Hb. U.S. 41940); Tennessee Pass, Lake County, July 10, 1902, *George E. Osterhout* 2645 (Hb. N.Y.; type of *Taraxacum leiospermum* Rydb.); Gray's Peak, vicinity of, alt. 12000 ft., August 1882 and 1885, *Patterson* and *Beaty* (Hb. Field 209706); Georgetown, vicinity of, June 28–August 7, 1875, *Harry N. Patterson* (Hb. Field 208950); Cuchara River, below Laveta, alt. 2100 m., May 28, 1900, *Rydberg* and *Vreeland* 5540 (Hb. Greene 48459); South Park, July 1873, *John Wolf* 268 (Hb. U.S. 41954); Central Colorado in 1873, *idem* 669 (Hb. Field 211601).

New Mexico: Santa Fe Canyon, 9 miles east of Santa Fe, alt. 8000 ft., June 2, 1897, *A. A.* and *E. Gertrude Heller* 3642 (Hb. Greene 48457; Hb. U.S. 306394; the basis, as to material examined and not as to synonomy Waldst. and Kit., of the name *Taraxacum lividum* Heller); Pecos River National Forest, at Winsor's Ranch, alt. 8400 ft., June 29, 1908, *Paul C. Standley* 4022 (Hb. U.S. 498416); Cloudcroft, June, 1912, *Elmer Stearns* 356 (Hb. U.S. 691021); Cloudcroft, vicinity of, June 30, 1899, *E. O. Wooton* (Hb. U.S. 739580 and 739583); Cox Canyon, Sacramento Mts., August 9, 1899, *idem* (Hb. U.S. 562510, 735339, and 739582); Silver Spring Canyon, Sacramento Mts., July 6, 1899, *idem* (Hb. U.S. 739581); Winter Folly, Sacramento Mts., August 13, 1899, *idem* (Hb. U.S. 735338).

Alaska (and neighboring islands): Fort St. Michaels, Norton Sound, June 23, 1865–1866, *H. M. Bannister* (Hb. Cornell Univ.; Hb. Field 301948); St. Paul Isl., July 9, 1899, *L. J. Cole* (Hb. U.S. 376691); Kadiak, July 2, 1899, *idem* (Hb. U.S. 376690); Kukak Bay, July 1–5, 1899, *Coville* and *Kearney* 1524 and 1690 (Hb. U.S. 376708 and 376711); Hall Island, July 14, 1899, *iidem* 2028 (Hb. U.S. 376716); Unalaska, July 8, 1899, *iidem* 1721 (Hb. U.S.

376713); Attu Isl., June 26, 1873, *W. H. Dall* (Hb. U.S. 424065); Unalaska, July 11, 1892, *B. W. Everman* 69 (Hb. U.S. 376727); Dutch Harbor, Unalaska Isl., July 17, 1899, *B. E. Fernow* (Hb. Cornell Univ.); Johnson River, between Cook Inlet and the Tanana River, June 27, 1899, *E. F. Glenn* (Hb. U.S. 376755; type material of *Taraxacum mutilum* Greene); Iliamna River, Lake Iliamna region, open woods, June 29, 1902, *M. W. Gorman* 80 (Hb. U.S. 420101); Copper Center, vicinity of, in 1908, *C. W. H. Heideman* 78 (Hb. U.S. 421973); Unalaska, *A. Kellogg* 301 (Hb. U.S. 424067 and 424068); Popof Isl., Shumagin Isls., July 8–19, 1899, *Trevor Kincaid* (Hb. U.S. 376794); St. Matthew Isl., August 11, 1891, *James M. Macoun* (Hb. U.S. 249296); St. Paul Isl., August 3, 1891, *idem* (Hb. Can. 20478); St. Paul Isl., dampish banks, July 13, 1896, *idem* (Hb. Can. 20479); St. Paul Isl., grassy banks, July 1897, *idem* (Hb. Can. 20481; labeled "*Taraxacum Chamissonis*, Greene typical" in Greene's own handwriting); St. Paul Isl., June 23–August 7, 1914, *idem* (Hb. Can. 94004); Kodiak Isl., crevices of rocks, May 31, 1897, *idem* (Hb. Can. 16754); Hall Isl., crevices of rocks, August 11, 1891, *idem* (Hb. Can. 20621); Unalaska, July 4, 1896, *idem* (Hb. Can. 16755; labeled typical *T. Chamissonis* by E. L. GREENE); Valley of Alatna River, about 15 miles above its mouth, July 20, 1901, *W. C. Mendenhall* (Hb. U.S. 377350); St. Paul Isl., August 4, 1891, *C. Hart Merriam* (Hb. U.S. 424071); Kenai, June 9, 1901, *H. P. Nielsen* 11 (Hb. U.S. 378436); St. Paul's Island, July 19, 1890, *Wm. Palmer* 304 (Hb. U.S. 327969); Kodiac, July 28, 1904, *C. V. Piper* 4231 (Hb. U.S. 420683); Kenai, August 18–20, 1904, *idem* 4228 (Hb. U.S. 420680); Unga Isl., Shumagin Isls., July 12–14, 1899, *DeAlton Saunders* (Hb. U.S. 376801); Adakh Isl., July 1, 1893, *C. H. Townsend* (Hb. U.S. 219332); St. Paul Isl., August 14, 1895, *True* and *Prentiss* 82 (Hb. U.S. 231549); Tuksuk Channel, vicinity of Port Clarence, rocky banks, August 5, 1901, *F. A. Walpole* 1746 (Hb. U.S. 378852); Cape Espenberg, lat. 66° 38′ N., long. 163° 46′ W., July 28, 1894, *James T. White* (Hb. U.S. 270305); St. Lawrence Isl., August 27, 1894, *idem* (Hb. U.S. 270328).

Yukon: Canyon of the Upper Liard River, lat. 60°, June 26, 1887, *Dawson* (Hb. Can. 15119; type of *Taraxacum lacerum* Greene); Coral Creek Hill, Yukon River, June 29, 1893, *Frederick Funston* 101 (Hb. U.S. 370774); Herschell Isl., lat. 69° 35′ N., long. 139° W., August 1914, *Frits Johansen* 233 (Hb. Can. 98717; Hb. Field 483379); Five Finger Rapids, July 4, 1899, *J. B. Tarleton* 72 (Hb. U.S. 391518).

British Columbia: Mt. McLean, near Lillooet, alt. 7000 ft., July 29, 1916, *J. N. Macoun* (Hb. Can. no. 98692 in Hb. Field, 483391); Mt. McLean, alt. 6500 ft., July 29, 1916, *idem* (Hb. Can. no. 98693 in Hb. Field, 483392); Mt. McLean, alt. 6300 ft., July 29, 1916, *idem* (Hb. Can. 98694); Mt. McLean, alt. 6000 ft., July 29, 1916, *idem* (Hb. Can. 98695); Mt. McLean, along irrigation ditch, alt. 5000 ft., July 3, 1916, *idem* (Hb. Can. no. 98696 in Hb. Field, 483393); Mt. McLean, alt. 6500 ft., July 22, 1916, *idem* (Hb. Can. no. 98697 in Hb. Field, 483394); Mt. McLean, alt. 5500 ft., July 19, 1916, *idem* (Hb. Can. no. 98699 in Hb. Field, 483395); Whipsaw Creek, west of

Princeton, July 24, 1905, *idem* (Hb. Can. 77000); Yale, grassy slopes, May 17, 1889, *John Macoun* (Hb. Can. 15120); Spence's Bridge, damp grassy places, May 28, 1889, *idem* (Hb. Can. 15130); Fraser River, west of, damp grassy places, June 10, 1875, *idem* (Hb. Can. 15121); Kicking Horse Lake, Rocky Mts., July 18, 1885, *idem* (Hb. U.S. 219795).

Alberta: Jasper Park, at Shovel Pass, low ground near a brook, alt. 6000–6500 ft., August 20, 1918, *James M. Macoun* (Hb. Can. nos. 98686, 98687, and 98688 in Hb. Field, 483386, 483387, and 483388, respectively); Island Creek, north of Peace River, July 15, 1903, *idem* (Hb. Can. 61240); Bragg's Creek, foothills south of Calgary, July 16, 1897, *John Macoun* (Hb. Can. 22776); Calgary, 3 miles west of, along railroad, June 7, 1897, *idem* (Hb. Can. 22792); Banff, swamps, June 27, 1891, *idem* (Hb. Can. 15127); St. Ann, June 9, 1898, *W. Spreadborough* (Hb. Can. 19744).

Utah: Uintah Mts., above Bear River, alt. 12000 ft., August 1869, *Sereno Watson* 723 (Hb. U.S. 41937); Marysvale, alt. 6000 ft., May 21, 1894, *Marcus E. Jones* 5338 (Hb. U.S. 326832; a very unique specimen with exterior bracts of involucre greatly elongated and almost equal to the interior bracts, the flowering head over 5 cm. wide).

California: Bear Valley, San Bernardino County, alt. 6500 ft., June 3, 1901, *S. B. Parish* 4977 (Hb. U.S. 414859).

Besides the specimens cited, I have examined a number from the locality (Kamchatka; also Bering Island, Commander Islands, etc.) whence LEDEBOUR obtained his type. Most of the material from that vicinity, from the Aleutian Islands, and from Alaska proper, has the outer bracts tending to be rather short, ovate, and notably blackish when dried, with the scarious margins highly distinct. This character is not constant, however, and there are numerous variations seen. South of Alaska, nearly every specimen examined has longer, more lanceolate bracts, which tend to remain pale or dark green when dried. Even here, however, there are some marked exceptions to the rule. Thus, for example, *Standley* 4022 from New Mexico (Hb. U.S. 498416) has the dark, scarious-margined, ovate outer bracts typical of the Alaskan material.

GREEN (Pittonia 4:228. 1901), writing upon *Taraxacum* in North America, named the Bering Sea form *T. Chamissonis*.[7]

[7] While GREENE cited no type, many of the Bering Sea specimens listed (in Hb. Can. and Hb. U.S.) had been examined by him and are labeled *T. Chamissonis* in his own handwriting. As noted, the specimen by *J. M. Macoun* from St. Paul Island (Hb. Can. 20481) had been labeled "typical" by him and may be regarded as being practically type material.

He stated that "its most constant peculiarity is that of a very dark-colored, almost blackish, involucre, of which the outer scales are very broad, strictly erect, and imbricated." Reference to LEDEBOUR'S work, however, shows that this was essentially the form which LEDEBOUR described from Kamchatka as *T. ceratophorum* ("squamis omnibus erectis; exterioribus lato-lanceolatis, nigricantibus" etc.),[8] hence *T. Chamissonis* is to be regarded as typical *T. ceratophorum*.

T. lacerum Greene and *T. mutilum* Greene are plainly mere foliage forms of *T. ceratophorum*. The type sheet of *T. lacerum* (in Hb. Can.) bears four small plants. These are not noticeably different from ordinary *T. ceratophorum* except as to the unique leaves,[9] which consist only "of a linear rachis-like body and a few pairs of divaricate or retrorse subulate-linear or falcate lobes." The bracts are highly ceratophorous. I have not been permitted to examine the type of *T. mutilum* (in Hb. Mo. Bot. Gard.), but an excellent cotype, previously cited, is in the U.S. National Herbarium.[10] This has leaves slightly less reduced than in *T. lacerum*, but bracts practically as corniculate. It is matched very closely by *J. M. Macoun's* plant from Churchill, Hudson Bay (Hb. Can. 79286), and, somewhat less closely, by *White* and *Schuchert* 110 from Baffin Land. The discontinuous distribution indicated by the four collections (*T. lacerum* from northern boundary of British Columbia, *T. mutilum* from Johnson River in Alaska, from along Hudson Bay, and from Baffin Land), suggests that either these forms represent one valid species of highly interrupted range or else they are merely foliage forms of *T.*

[8] Fl. Altaica 4:149. 1833. In his still earlier work (Icon. pl. Fl. Ross. 1:9. 1829), LEDEBOUR gave only an abridged description: "L. anthodii squamis erectis infra apicem longe corniculatis; exterioribus lato-lanceolatis; interioribus lanceolatis, foliis runcinato-sinuatis: laciniis inaequalibus; majoribus subtriangularibus. Hab. in Kamtschatka. 4 Fl. Majo, Junio." His accompanying plate (*pl. 34*) is somewhat crude and shows the outer involucre spreading above the middle and consisting of narrowly lanceolate or even linear bracts. Apparently LEDEBOUR himself had noticed this discrepancy; for in his later description in the *Flora Altaica*, not only did he retain the character "lato-lanceolatis" for the outer bracts, but he actually inserted the word "omnibus" to qualify "squamis erectis."

[9] These resemble very closely those figured by HANDEL-MAZZETTI (Monogr. Taraxacum, *pl. 5. fig. 2.* 1907) for *T. balticum*, a species unknown to me.

[10] Indeed, GREENE himself had even written "type" upon the label of this specimen, although in his description he listed Hb. Mo. Bot. Gard. as containing the type.

ceratophorum. Touching this point, a parallel study of *T. lyratum* is very illuminating. In several cases I have seen among material that was positively *T. lyratum* a freakish foliage form that looked superficially just like *T. mutilum*. In fact one of these specimens (*Walpole* 1987, Hb. U.S. 379107) appears to have deceived GREENE, for he had labeled it *T. mutilum*. Inasmuch as true *T. lyratum* is seen thus to produce a similar foliage form at times, and since true *T. ceratophorum* is known to be present wherever *T. mutilum* or *T. lacerum* has been collected, there seems to be no reason for considering either *T. mutilum* or *T. lacerum* distinct from *T. ceratophorum*. At the most they evidently can rank no higher than mere forms or varieties.[11]

Many older specimens have been determined in herbaria, some by ASA GRAY, as *T. montanum* Nutt. (non Mey. et DC.), a species cited by NUTTALL from "on the banks of the Platte, in subsaline situations toward the Rocky Mountains, and in the highest valleys of the Colorado of the West." This name was retained by WOOTON and STANDLEY (Contr. U.S. Nat. Herb. **19**:627. 1915) notwithstanding the validity of the previous name *T. montanum* (Mey.) DC. RYDBERG (Fl. Rocky Mts. 1035. 1917), however, recognizing the impropriety of retaining NUTTALL'S duplicating name, created the new and similar name (*Leontodon*) *monticola*, which thus is directly equivalent by synonomy with NUTTALL'S species. Even if NUTTALL'S species had been taxonomically worthy, however, which it was not, RYDBERG'S new name for it would be invalid, as GREENE (*loc. cit.*) had already created the name *T. dumetorum* for material which came from the same region and which did not specifically differ.[12] Obviously GREENE'S name would have

[11] It may be noted, however, that HANDEL-MAZZETTI (Monogr. Taraxacum 87. *pl. 5. fig. 2.* 1907) separates an apparently corresponding form of Europe, *T. balticum*, from the broader leaved *T. paludosum* (cf. footnote 9).

[12] WOOTON and STANDLEY, and also RYDBERG do in fact present *T. dumetorum*, which they have sought to differentiate as a separate species. I have examined all the types (in Hb. Greene) and other specimens cited for *T. dumetorum* by GREENE, and can find no differences other than those that can be proved to be field variations, or that would pass with the great majority of taxonomists as typifying merely inconstant forms. NUTTALL'S description, "caliculum biserial, short and appressed, the scales ovate or lanceolate, with broad membranaceous margins; sepals not corniculate, about twelve" shows that his plant was the form later treated by HANDEL-MAZZETTI as *T. lapponicum* Kihlm. In NUTTALL'S plant the bracts were thus not corniculate, whereas in typical *T. dumetorum* cornicula are present. These distinctions, however, do not appear to be of any value specifically.

preference. Yet even here we are confronted with difficulty, since the *T. dumetorum* type specimens (from Dale Creek, Wyoming, in Hb. Greene) are clearly a mere form or variety of true *T. ceratophorum*. Indeed, an additional "quite typical" specimen cited by GREENE (*Williams* 434) had once been listed by RYDBERG himself (Fl. Montana 484. 1900) as *T. ceratophorum*. Why he later abandoned the name (vide Rydb., Fl. Rocky Mts. 1034–1035. 1917) is not clear. As already stated, the American specimens from points south of Alaska (as also many from Alaska itself) tend to have external bracts somewhat different from those of Bering Sea (that is, typical) material. These exterior bracts vary from dark to light, from short to long, from ovate to lanceolate, from corniculate or widely dilated-bifid at apex to ecorniculate and acute, from appressed to spreading.[13] Occasionally they are as long as the inner bracts. Sometimes both sets of bracts are apically dilated, sometimes only the outer or inner set. Viewed in the light of these facts, *T. dumetorum* is seen to be synonymous with *T. ceratophorum*.

HANDEL-MAZZETTI (*loc. cit.* 73), in dealing with *T. ceratophorum*, makes a singular segregation of specimens under the separate binomial *T. lapponicum* Kihlm. The range given is essentially the same as recognized by him for *T. ceratophorum*. The chief diagnostic distinction relied upon appears to be the ecorniculate character of the bracts. It is with reluctance that I am compelled to reject his treatment.[14] The species concept and "species sense" of one who, like HANDEL-MAZZETTI, has surveyed the entire genus for all the regions of the world, are naturally and very properly entitled to high respect, but the variations in the corniculate character of the bracts are so great in North American specimens as to render illogical and really impossible any such differentiation (cf. footnote 12). It does not also appear that we even have two parallel series, connected, as stated by HANDEL-MAZZETTI, with each other by numerous intermediate

[13] In one specimen from the type locality of *T. ceratophorum* (*C. Wright*, Petropaulovski, Kamchatka, 1853–1856, Hb. U.S. 424073), the outer bracts are lanceolate and their margins are scarious only to a very slight degree.

[14] At least as to North American plants. As to the status of *T. lapponicum* Kihlm. in Europe, I have seen too few specimens to judge accurately.

forms.[15] The *lapponicum* form is much less abundant and appears to be merely an offshoot from *T. ceratophorum*. Sometimes, however, especially in the northeastern part of the continent, it passes into *T. vulgare*.[16] FERNALD and ROBINSON (Gray's *Manual*, ed. 7. 865. 1908) evidently included some of these transitional forms in their *T. officinale* var. *palustre* Blytt, from "eastern Quebec to Connecticut." At the time true *T. ceratophorum* was unknown to them from New England (cf. FERNALD, Rhodora **4**:155. 1902), but since then it has been discovered by PEASE (Rhodora **19**:111 and 221. 1917) in New Hampshire; and many years before a specimen was collected by ROBBINS.[17] The true *T. officinale* var. *palustre* (*T. paludosum* [Scop.] Schlecht.) is not cited for North America by HANDEL-MAZZETTI.[18]

T. leiospermum Rydb., from Colorado, is found to differ from the ordinary *T. ceratophorum* merely in having slender ecorniculate bracts and a slightly greenish tint to the brown, less muricate achenes. In HANDEL-MAZZETTI'S treatment *T. leiospermum* would belong, more precisely, with *T. lapponicum*. Of all the many specimens that I have studied, I have found no other specimen exactly matching RYDBERG'S type (in Hb. N.Y.) in the smoothness and color of the achenes. My failure in this respect suggests that the type was merely one of the excessively numerous forms conspicuous in this genus, which apparently often are

[15] "In der ganzen Zone der Gebirge des westlichen Nordamerika ist *T. ceratophorum* mit *T. lapponicum* durch zahlreiche Formen verbunden, die in den Merkmalen der Hüllblättchen Zwischenstellungen einnehmen," *loc. cit.* 66.

[16] HANDEL-MAZZETTI (*loc. cit.* 84) gives an exhaustive treatment of numerous forms intermediate between *T. vulgare* and *T. paludosum*, the latter being a species very close to *T. lapponicum*. He cites none for America, however.

[17] I have not seen this specimen. It was found in the herbarium at Berlin by HANDEL-MAZZETTI, and was determined by him as *T. lapponicum*.

[18] At various times some of our foremost American botanists have used the names *palustre* and *alpinum* for American specimens of *T. ceratophorum*. The real *T. paludosum* (Scop.) Schlecht and *T. alpinum* (Hoppe) Heg. and Heer, dating back originally to 1772 and 1821 respectively, are not given by HANDEL-MAZZETTI for North America. While I have been unable to examine enough European material to permit of definite conclusions, it would seem that the two species are too close together. In any case, it appears certain that if American forms of *T. ceratophorum* with ecorniculate bracts are to be segregated, they must be referred to *T. paludosum* or *T. alpinum*, rather than to the more recent *T. lapponicum*.

perpetuated here and there through parthenogenetic reproduction.[19]

Taraxacum lividum Heller (exclud. synon. Waldst. et Kit.) is seen, from the specimens cited (*A. A.* and *E. G. Heller* 3642), to be likewise a form of *T. ceratophorum*. Most of the bracts are ecorniculate, thus placing the plants, in HANDEL-MAZZETTI's treatment (*loc. cit.* 74), with *T. lapponicum*.

3. TARAXACUM ERIOPHORUM Rydb., Fl. Montana, Mem. New York Bot. Gard. 1:454. 1900 (non Schott ex Tchihatcheff, Asie mineure 3^2:372. 1860; nomen nudum quod=*T. syriacum* Boiss., fide Handel-Mazz., Monograph. Taraxacum 162. 1907); *T. ovinum* Greene, Pittonia 4:229. 1901; *T. angustifolium* Greene, *loc. cit.*; *T. ammophilum* Nels. ex Greene, *loc. cit.* 233. *L. eriophorum* Rydb., Fl. Rocky Mts. 1035. 1917, *L. angustifolium* Rydb. *loc. cit.*; *L. ammophilum* Rydb., *loc. cit.* Pl. XXXIII.

Herba polymorpha, nunc pumila et rosulata (forma descriptionis orig.), nunc robustior, 3–8 (etiam −30) cm. alta. Radix et folia et scapi eis *T. ceratophori* non conspicue dissimiles, foliis autem saepius membranaceis et pallidis, rarius profunde pinnatifidis, iuvenilibus raro longe lanuginosis versus basim. Capitula 1.5–2.5 cm. alta et paulo latiora. Involucrum pallidum vel atroviride. Involucri foliola plerumque ecorniculata vel rarissime ad apicem dilatato-corniculata et plus minusve atrata, exteriora adpressa vel minime patentia, interiorum longitudinis 0.2–0.6 aequantia, 4–15 mm. longa, margine plus minusve distincte decolorato. Flores vivi ad anthesin non observati. Achaenia 4–5 mm. longa, rufa rufopurpureave, supra tuberculis angustis vel spinulis dense obsita, saepe acute tetragona, in cuspidem crassam vel angustam, et brevem vel quartae parti totius fructus aequantem cuneate attenuata. Rostrum tenue, achaenio paulo vel multo longius. Pappus albus, 4–8 mm. longus.

DISTRIBUTION.—Alberta to Wyoming; a form with highly corniculate bracts occurs in Alaska.

SPECIMENS EXAMINED.—Alberta: Morley, meadows, etc., June 12, 1885, *John Macoun* (Hb. Can. 15117); Laggan, June 28, 1905, *idem* (Hb. Can. 65618 and 65619); Waterton Lake, Sheep Mt., July 31, 1895, *idem* (Hb. Can. 11711, type of *T. ovinum* Greene; Hb. Greene 48435).

[19] Concerning the fixation of new colors in *Taraxacum* achene coats through the operation of parthenogenesis, *cf.* footnote 24.

Alaska: Vicinity of Port Clarence, gravel flats near beach, Teller Reindeer Station, September 3, 1901, *F. A. Walpole* 1980 (Hb. U.S. 379098).

British Columbia: Kicking Horse Lake, Rocky Mountains, springy places, July 20, 1885, *John Macoun* (Hb. Can. 15128; Hb. Field 227895).

Montana: Sheridan, in 1892, *Mrs. L. A. Fitch* (Hb. Mont. Agric. Exper. Sta.; type); Anaconda, mountain swales, alt. 6000 ft., May 20, 1906, *J. W. Blankinship* 723 (Hb. Can. 73794; Hb. Field 225568; Hb. U.S. 541188).

Wyoming: Dale Creek, July 1, 1896, *Edward L. Greene* (Hb. Greene 48449, 48450, and 48451; the three type sheets of *T. angustifolium* Greene); Pole Creek, June 2, 1894, *Aven Nelson* 109 (Hb. U.S. 284425); Horse Creek, June 9, 1894, *idem* 205 (Hb. Field 432099; Hb. U.S. 284424); Sand Creek, Albany Co., May 31–June 1, 1900, *idem* 6987 (Hb. Greene 48427, type of *T. ammophilum* Nelson ex Greene; Hb. U.S. 433375); Sand Creek, Albany Co., June 1, 1900, *idem* 6988 ex parte (Hb. U.S. 433376).

The specimens originally distributed by NELSON (no. 6987) as *T. ammophilum* are rather small, averaging mostly under 1 dm. in height, and are of a pallid, somewhat glaucous appearance. Their achenes, when mature, are distinctly reddish, as in *T. laevigatum*. The involucral bracts are almost entirely without dilations at the apex. Except for the achenes, the plants match perfectly some plants considered by HANDEL-MAZZETTI as *T. lapponicum* Kihlm., but regarded by myself as a form or variety of *T. ceratophorum*. They are in no way referable to the European *T. laevigatum*, as suspected by HANDEL-MAZZETTI (*loc. cit.* 110), who appears never to have seen NELSON's specimens.

Some of the material examined is darker green, but otherwise identical. *Blankinship* 723 from Montana, consisting mostly of immature specimens, is an example of this. The Blankinship plants are particularly instructive, further, in showing the aspect of immature and dwarfed plants. Some of these (for example, Hb. Field 225568) match exactly RYDBERG's three tiny immature type specimens of *T. eriophorum* (in Hb. Mont. Agric. Exper. Sta.).[20] RYDBERG did not describe the achenes, since there were no mature ones present.[21] The immature achenes of the Blankinship collection are brown, as in RYDBERG's type material, but the

[20] BLANKINSHIP's plants were collected at Anaconda, a distance of only 55 miles (90 km.) from Sheridan, whence the type of *T. eriophorum* came.

[21] The name *eriophorum* alluded to the brown hairs found on the small type plants, but this character is entirely inconstant in this species and has no real taxonomic value.

nearly mature ones (for example, Hb. U.S. 541188) are distinctly reddish. Thus RYDBERG's type plants are seen to be connected perfectly with the type material of *T. ammophilum*, and, from priority, the name *T. eriophorum* must have the preference.

The type material of *T. ovinum* Greene, from Alberta, consists of several small, more or less dwarfed and immature specimens. The achenes in the oldest head found (in Hb. Can.), while not yet very reddish, have the acutely tetrangular shape that I have observed in numerous other mature specimens of *T. eriophorum*. The involucre, although sometimes duplicated by *T. ceratophorum*, is more typical of *T. eriophorum*, and there remains no doubt that *T. ovinum* is purely synonymous with *T. eriophorum*.

T. angustifolium Greene was founded upon three specimens from Dale Creek, Wyoming. The leaves and scapes are much better developed than in *T. ovinum*, the scapes reaching a height of over 2.5 dm.; but the technical characters of the head are essentially the same. Moreover, the numerous mature achenes are definitely reddish in color. GREENE (*loc. cit.* 232) termed their color "chestnut brown," but inaccurately so, for the color is fully as reddish as in many genuine specimens of the red-achened *T. laevigatum*. The leaves are rather long, slender, and graceful, but certainly do not serve to separate the plants specifically from true *T. eriophorum*.[22]

HANDEL-MAZZETTI (*loc. cit.*) has omitted *T. eriophorum* Rydb. entirely from his monograph, and it is evident that he was entirely unfamiliar with it. The species is closely parallel with *T. ceratophorum*, from which it differs in having red achenes and in having the bracts much more often slender and without dilated tips. One might wonder whether it may be only a form of *T. ceratophorum* in which the achenes are red. Various investigators have shown that apogamy or parthenogenesis is frequent in *Taraxacum*.[23] SCHKORBATOW (Entwickelungsgeschichtliche Stud. an *Taraxacum officinale* Wigg., Bot. Institut. Charkow, p. 50. 1910) also states

[22] Almost the exact counterpart to this foliage is sometimes observed in a form of *T. ceratophorum* (for example, *Mendenhall*, Valley of Alatna River, Hb. U.S. 377350).

[23] For references to the experiments and observations of RAUNKIAER, MURBECK, JUUL, and others, see IKENO, Ber. Deutsch. Bot. Gesells. **28**:394. 1910.

that various colors of achenes may thus become fixed and hereditary.[24] Whether, however, the colors will remain fixed in the achenes of all the plants of a locally generated race upon a recurrence of normal fertilization (with attendant lapse of apogamy) is doubtful. Surely subsequent cross-pollination with specimens from the antecedent stock might be expected to occur and to result, at times, in a repetition of the former achene color. In any case, the observable tendency of the achenes of *T. eriophorum* to be more sharply tetragonal and of the bracts to be undilated at the apex in a much higher percentage of specimens, makes it seem that *T. eriophorum* is not a red-fruited variety of *T. ceratophorum*, but is rather a distinct species.

The Alaskan specimen by *Walpole* (no. 1980, Hb. U.S. 379098) has slender elongate leaves, much as in the types of *T. angustifolium*, and its achenes are bright red. The involucral bracts, however, especially the inner ones, are exceedingly corniculate, much as in the extremely corniculate forms of *T. ceratophorum*.[25]

4. TARAXACUM VULGARE (Lam.) Schrank, Primit. Fl. Salisburg. 193. 1792; *Leontodon Taraxacum* Linn., Sp. Pl. **2**:798. 1753 (diagnose incompl. fide Handel-Mazz.); Pollich, Hist. plant. Palatin. **2**:379. 1777; *L. vulgare* Lamarck, Fl. Françoise **2**:113. 1778; *T. officinale* Weber, Prim. Pl. Holst. 56. 1780; Roth, Tentam. Fl. Germ. **2**²:147. 1793; *T. Dens-leonis* Desf., Fl. Atlant. **2**:228. 1800 (fide Indicis Kew., locum cit. non vidi); *T. latilobum* DC., Prodr. **7**:146. 1838; *T. mexicanum* DC., *loc. cit.*; *T. officinale* var. *palustre* Fernald and Robinson, Gray's Man., ed. 7, p. 865. 1908 (forsan non [Smith] Blytt, Bentham, et al.); *T. paradoxum* Somes, Amer. Botanist **15**:27. 1909; *L. latilobum* Britton, Britt. and Brown Ill. Fl. N. Amer., ed. 2, **3**:315, *fig. 4063*. 1913; *T. minus*

[24] "In der Natur findet man verschiedene Farben-Schattierungen an den *Taraxacum*-Früchten, von dunkelbraun bis hellgrünlich: die ausgesprochenen Färbungen in typischen Modificationen genommen (rein hellgrün und rein dunkelbraun) werden als solche durch Vererbung fixirt." (L. SCHKORBATOW, *loc. cit.* For English summary of SCHKORBATOW'S work, see CHAMBERLAIN, BOT. GAZ. **52**:167. 1911.)

[25] To those who accept HANDEL-MAZZETTI'S differentiation of North American material between *T. ceratophorum* and *T. lapponicum*, this specimen will appear correspondingly distinct from *T. eriophorum*. I have found no such intermediate forms in *T. eriophorum*, respecting dilations of the bract tips, as are abundant in *T. ceratophorum*. Nevertheless, there seems insufficient evidence at hand to warrant proposing *Walpole's* 1980 as the type of a new species.

Lon. et var. *subscaposum* Lunell (ex synon. *L. Taraxacum* Britton, etc.), Amer. Midl. Nat. **5**:31. 1917; *L. mexicanum* Rydb., Fl. Rocky Mts. 1034. 1917.

Herba plerumque maiuscula, 5–50 cm. (rarissime "–1.20 m.") alta. Radix crassa, simplex vel multiceps, fusce corticata, collo vix squamato, large lanigero vel raro glabro. Folia nunc terrae adpressa, nunc suberecta, viridia, plerumque infra et in nervo medio sparse pilosa vel rarius glaberrima, plerumque ampla, plus minusve oblanceolata (7 mm. –15 cm. lata), acuta vel obtusa, versus basim brevius longiusve angustata, rarius large dentata tantum, plerumque autem variis modis, interdum usque ad nervum medium, runcinato-incisa, lobis latius angustiusve triangularibus vel rarius linearibus, integris vel dentato-fissis, recurvis, saepe lobulis minoribus interiectis, lobo terminali plerumque maiore: Scapi numerosi vel raro singuli, erecti vel adscendentes, crassi (2–7 mm.), florendi tempore sub capitulo saltem longe lanigeri, denique raro glabri, floriferi foliis ±aequilongi, rarius multo breviores vel multo longiores. Capitula magna (solum in speciminibus depauperatis parva), circum 2–2.5 cm. longa et aperta latitudine multo maiore. Involucri foliola numerosa, utriusque seriei ±15–20, griseo-viridia, raro atrata, interdum leviter pruinosa, ecorniculata vel raro corniculis parvis vel rarissime maioribus instructa, linea dorsali fusca nulla. Exterioris seriei foliola interioribus vix latiora, sed paulo breviora, inter se fere aequilonga, iam in alabastris adultioribus supra basim reflexa, vel raro patula vel unum alterumve eorum vel rarissime plurima semper erecta, linearia (1.3–3 mm. lata et 12–14 mm. longa) vel rarissime latiora, margine raro indistincte decolorato. Flores numerosissimi, lutei vel raro subpallidiores, involucro circum 5–10 mm. longiores. Achaenia parva, 3–4 mm. longa, pallide griseo- vel olivaceo-brunnea, supra tuberculis mediocribus longioribusve dense obsita, in cuspidem cylindricam longiusculam et tenuem vel brevissimam et crassam, totius fructus sextam vel rarius tertiam fere partem metientem abruptissime contracta. Rostrum tenue, achaenio duplo vel plus triplo longius. Pappus albus, 6–8 mm. longus, rostro brevior.

DISTRIBUTION.—Labrador and North Carolina to Alaska, California, and Mexico, and elsewhere almost throughout the world; indigenous (fide Handel-Mazz.) in meadows of Europe and Western Asia.

SPECIMENS EXAMINED.—Labrador: Rama, August 20–24, 1897, *J. D. Sornberger* 64*x* (Hb. U.S. 411050; a form with the leaves lanceolate to spatulate and not deeply incised, some of them merely denticulate).

Newfoundland: Hermitage Bay, vicinity of Balena, June 16, 1903, *William Palmer* 1365 (Hb. U.S. 492202).

Quebec: Mt. Albert, Gaspe County, by alpine brooks or in crevices of wet hornblende schist, alt. 600–1075 m., July 20, 1906, *Fernald* and *Collins* 263 (Hb. U.S. 606098); Mt. Albert, Gaspe County, meadows and fields, also on mountains, August 19, 1882, *John Macoun* (Hb. Can. 15113); Salt Lake, Anticosti Isl., pastures and fields, August 11, 1883, *idem* (Hb. Can. 15105); Orono and vicinity, fields, September 1890, *F. L.* and *LeRoy H. Harvey* 579 (Hb. U.S. 606242); Orono, June 2, 1897, *P. L. Ricker* 233 (Hb. U.S. 414356); St. Francis River, at Boundary Lake, August 14, 1902, *W. W. Eggleston* (Hb. U.S. 492531).

Massachusetts: Middleboro, May 14, 1901, *Joseph Murdoch* (Hb. Field 471888); Middleboro, May 14, 1901, *Richard Murdoch* (Hb. Field 472180).

Rhode Island: Cumberland, railroad embankment, May 9, 1900, *E. B. Chamberlain* 68 (Hb. U.S. 491069).

New York: Chemung County, roadsides and fields, May 19, 1893, *T. F. Lucy* 14529 (Hb. Field 5306); Cold Spring Harbor, Long Island, waste places, August, 1903, *H. N. Whitford* 20 (Hb. Field 144122).

Pennsylvania: Westtown Farm, Chester County, May 26, 1905, *S. P. Hadley* 1 (Hb. U.S. 646339); Ephrata, vicinity of, May 14, 1900, *A. A. Heller* (Hb. Field 430006; Hb. U.S. 407015); Conestoga Creek, east of Lancaster, *idem*, April 28, 1900 (Hb. U.S. 407016; form with finely divided leaves); Conestoga Creek, Lancaster, May 2, 1890, *John K. Small* (Hb. Field 168088 and 168089); Harrisburg, June, 1887, *idem* (Hb. Field 168174); Conewago, vicinity of, May 14, 1891 (Hb. Field 167805 and 167810).

District of Columbia: without locality, in 1863, herb. M. S. Bebb (Hb. Field 17549).

Virginia: Louden County, August 1888, *Jesse H. Holmes* (Hb. U.S. 41946 and 41948); Chatham Hill Gap, Walker Mountain, Smyth County, alt. 3000 ft., June 13, 1892, *John K. Small* (Hb. Field 390271); White Top Mountain, Smyth County, alt. 4000–5000 ft., May 28–29, 1892 (Hb. Field 390272).

West Virginia: Pickens, June 24, 1908, *Huron H. Smith* 1364 (Hb. Field 241895).

North Carolina: Roan Mountain, September 1, 1902, *W. A. Cannon* 223 (Hb. U.S. 510188).

Ontario: Kingston, May 27, 1897, *J. Fowler* (Hb. Field 83469); Kingston, May 29, 1895, *idem* (Hb. U.S. 249777).

Ohio: Dayton, abundant and troublesome as a weed, May 25, 1904, *J. Lane Reed* (Hb. U.S. 444728 and 444729; a gigantic form escaped from cultivation, the leaves becoming, before end of fruiting period, over 4 dm. long and the scapes 7.75 dm. long); Chillicothe, in 1885, *H. T. Safford* 12 (Hb. U.S. 515462).

Michigan: Schoolcraft, uncleared ground, June 11, 1903, *A. B. Burgess* 129 (Hb. Field 141460).

Indiana: Mattsville, vicinity of, in open ground, May 10, 1892, *Guy Wilson* 19 (Hb. U.S. 228418); Mishawaka, June 1891, *E. B. Uline* (Hb. Chi. 260181).

Wisconsin: Green Bay, April, *J. H. Schuette* (Hb. Field 377994); Brown County, in yard, without date, *idem* (Hb. Field 377995).

Illinois: Evanston, dry field, July 4, 1919, *Earl E. Sherff* 3087 (Hb. Field 484462 and 484463), and in rich woods, July 4, 1919, *idem* 3088 and 3090 (Hb. Field 484464, 484465 and 484468, 484469 respectively); Urbana, open thicket, May 28, 1907, *Frank C. Gates* "1561:3" (Hb. U. S. 649050).

Minnesota: Fort Snelling, May–June, 1890, *E. A. Mearns* 161 (Hb. U.S. 649285 and 649286).

Iowa: Decatur County, pastures and waysides, common, May 28, 1896, *T. J.* and *M. F. L. Fitzpatrick* (Hb. Field 123803).

Missouri: Vulcan, railway tracks, May 8, 1908, *Huron H. Smith* 449 (Hb. Field 240920).

North Dakota: Grand Forks, vicinity of, in 1894, *C. A. Egebretson* 43 (Hb. Chi. 351987).

South Dakota: Mayo, meadows, June 20, 1914, *W. H. Over* 1828 (Hb. U.S. 582845); Rapid City, alt. 3700 ft., June 25, 1892, *Per Axel Rydberg* 846 (Hb. U.S. 211334).

Nebraska: Lincoln, May 10, 1886, *T. A. Williams* (Hb. U.S. 750371).

Kansas: Riley County, grassland, in 1896, *J. B. Norton* 748 (Hb. U.S. 353535).

Alberta: Jasper Park, Cabin Creek near Jasper, roadsides, June 15, 1918, *James M. Macoun* (Hb. Can. no. 98691 in Hb. Field, 483390).

Wyoming: Crow Creek, Albany County, moist banks, July 8, 1903, *Aven Nelson* 8905 (Hb. U.S. 581938); Yellowstone National Park, October 8, 1902, *Edgar A. Mearns* 4769 (Hb. U.S. 488386).

Colorado: Norwood Hill, San Miguel County, moist river banks, alt. 7000 ft., August 17, 1912, *Ernest P. Walker* 488 (Hb. U.S. 544606); Ouray, July 24, 1897, *C. L. Shear* 4102 (Hb. U.S. 858239).

New Mexico: Las Vegas, May 19, 1909, *T. D. A. Cockerell* (Hb. U.S. 660047); Rio Arriba County, hills south of Tierra Amarilla, alt. 2300 m., April 18–May 25, 1911, *W. W. Eggleston* 6545 (Hb. U.S. 660765); Tierra Amarilla, alt. 2280 m., April 18–May 25, 1911, *idem* 6594 (Hb. U.S. 660810); Raton, in streets, alt. 2100–2380 m., June 21–22, 1911, *Paul C. Standley* 6305 (Hb. U.S. 685335); Chama, vicinity of, along river, alt. 2380–2850 m., July 8, 1911, *idem* 6589 (Hb. U.S. 685611).

Utah: Milford, wet ground, June 4, 1902, *Leslie N. Goodding* 1039 (Hb. U.S. 485541); Provo, Wasatch Mts., June 16, 1902, *idem* 1156 (Hb. Field 215750); Big Cottonwood Canyon, below Silver Lake, June 29, 1905, *Rydberg* and *Carlton* 6455 (Hb. U.S. 508591); Wasatch Mts., abundant on plateau east of Ephraim Canyon, alt. 2900 m., August 14, 1907, *Ivar Tidestrom* 230

(Hb. U.S. 506794); Salt Lake City, alt. 5000 ft., May, 1869, *Sereno Watson* 722 (Hb. U.S. 41950).

Idaho: Pine, moist flat lands, August 16, 1910, *J. Francis Macbride* 619 (Hb. U.S. 542442); New Plymouth, "a terrible pest in lawns," July 14, 1910, *idem* 711 (Hb. Field 292597; Hb. U.S. 542478); Nez Perces County, along Hatwai Creek, April 24, 1892, *J. H. Sandberg* 42 (Hb. U.S. 243000); Hailey, common in empty lots, in 1909, *Woods* and *Tidestrom* 2762 (Hb. U.S.).

Nevada: Battle Mt., alt. 1350 m., July 23, 1913, *Albert E. Hitchcock* 626 (Hb. U.S. 765964); Jarbidge, along brook, July 12, 1912, *Nelson* and *Macbride* 2048 (Hb. U.S. 544856).

Alaska: Sitka, June 14-17, 1899, *Coville* and *Kearney* 804 (Hb. U.S. 376697); Wrangell, grassy hillside, May 6, 1915, Mr. and Mrs. *Ernest P. Walker* 617 (Hb. Field 466422); Wrangell, grassy slope, May 8, 1915, *iidem* 631 (Hb. Field 466435).

British Columbia: Oak Bay, vicinity of Sidney, Vancouver Isl., roadsides, April 22, 1913, *John Macoun* (Hb. Can. no. 98700 in Hb. Field, 483380).

Washington: Waitsburg, April 14, 1897, *Robt. M. Horner* 319 (Hb. U.S. 318829).

Oregon: Keno, alt. 4000 ft., May 9, 1898, *Elmer I. Applegate* 2015 (Hb. U.S. 361604); Umatilla National Forest, alt. 4300 ft., June 11, 1912, *C. L. Keithley* (Hb. U.S. 583213); Cottonwood Canyon, Malheur County, alt. 750 m., May 20, 1896, *John B. Leiberg* 2073 (Hb. U.S. 276280).

California: Mt. Shasta, north side of, alt. 5000–10,000 ft., June 15–30, 1897, *H. E. Brown* 442 (Hb. Field 412772); Amador County, March 23, 1896, *George Hansen* 1550 (Hb. Greene 48461).

Chihuahua: Chihuahua, vicinity of, alt. about 1300 m., June 5–10, 1908, *Edward Palmer* 353 (Hb. U.S. 573818).

Coahuila and Nuevo Leon: without locality, February–October, 1880, *idem* 761 (Hb. U.S. 41955).

San Luis Potosi: Alvarez, July 13–23, 1904, *idem* 180 (Hb. U.S. 471047).

Queretaro: Without locality, in 1910–13, *Agniel* 10535 (Hb. Field 484882).

Vera Cruz: Boca del Monte, March 13, 1894, *E. W. Nelson* 226 (Hb. U.S. 252392 *pro parte*); Las Vigas, June, 1893, *idem* 22 sub nomen *Senecio* (Hb. U.S. 252058).

Puebla: Chalchicomula, vicinity of, alt. 8000–8400 ft., March 15, 1894, *idem* 237 (Hb. U.S. 252392 *pro parte*).

Mt. Orizaba: without precise locality, July 25–26, 1901, *Rose* and *Hay* 5722 (Hb. U.S. 395506).

Mt. Popocatepetl: without precise locality, August 7–8, 1901, *iidem* 6067 (Hb. U.S. 395872).

Michoacan: Morelia, in streets, November, 1889, *Alfredo Duges* (Hb. U.S. 41956).

Mexico, civitate non cit.: *Berlandier* 849 (Hb. Boiss., cotype of *T. mexicanum DC.*).

Bermuda Isls.: Flatts, roadsides, August 16, 1913, *F. S. Collins* 314 (Hb. Field 464861); Agar's Isl., "not abundant," December 4, 1915, *idem* 430 (Hb. Field 464906).

Jamaica: Cinchona, alt. 4900 ft., in 1910, *Wm. Harris* 10926 (Hb. Field 294859).

As previously stated, *T. vulgare* tends to pass into *T. ceratophorum* in the northeastern part of North America. The *T. officinale* var. *palustre* of GRAY'S *Manual* (ed. 7, p. 865, *fig. 1015*. 1908) includes some of these transitional forms; so also does *T. latilobum* DC., collected originally in Newfoundland ("invol. squamis ecorniculatis, exter. patulo-reflexis . . . . proxime ad Dentem-leonis accedit," –DC., *loc. cit.*). *Murdoch* 1624, from Massachusetts (Hb. Field 470264) is typical of the GRAY'S *Manual* illustration, and yet is easily recognized as being true *T. vulgare*. *Fernald* and *Collins* 263 (Hb. U.S. 606098) from Quebec has the involucres fairly typical of *T. vulgare*, but in general habit it approaches *T. ceratophorum;* in fact, it was originally under the latter name. *Sornberger* 64*x* (Hb. U.S. 411050) from Labrador is still another form of *T. vulgare*. Its involucres are of the *T. vulgare* kind; but the foliage exactly matches that of *Fernald's* Grand River plant of Quebec (Hb. U.S. 605794), a plant that from involucral characters is seen however to be *T. ceratophorum*. Plants collected by *L. M. Turner* at Davie's Inlet, Labrador (Hb. U.S. 222755), have involucres clearly representing *T. vulgare*, but the foliage is very strange and is closer to that of *T. ceratophorum*, although not typical for that species. It seems entirely probable that a number of these intermediate forms are hybrids.

T. mexicanum DC. is retained as a valid species by HANDEL-MAZZETTI, who had seen at least nine specimens of BERLANDIER'S original type material, but I have seen no specimens of *Taraxacum* from Mexico that were not plainly *T. vulgare*. Even the excellent cotype specimen studied (in Hb. Boiss.) matches much of the *T. vulgare* material of the northern United States in foliage, in fruit, and in involucre. Nor does HANDEL-MAZZETTI'S description indicate any truly distinctive characters. Thus, for example, he describes the cusp of the achenes as being long in *T. mexicanum* and short or very short in *T. vulgare*, but there are numerous

specimens of genuine *T. vulgare* from various points all over North America in which the cusps are very long and slender, fully as much so as in any Mexican material studied by me. DE CANDOLLE himself was in doubt as to the validity of his species, even confessing that it was too close to *T. dens-leonis* (*T. vulgare*) and was perhaps only a variety.[26] HEMSLEY (Biol. Centr. Amer. **2**:261. 1881) regarded *T. mexicanum* as synonymous with our *T. vulgare* (*T. officinale*), and my own observations are in thorough accord with HEMSLEY'S treatment.

T. paradoxum Somes was admittedly a mere freak form of *T. vulgare*, having the stem foliate with alternate leaves, not scapose. The stems were bifurcate at the top. LUNELL'S *T. minus subscaposum* was likewise a mere leafy stemmed form ("caulis unifoliatus"). Such a form was not unknown before (cf. D. MCALPINE Bot. Atlas **1**: *pl. 25, figs. 6, 13b*. 1883).

5. TARAXACUM LAEVIGATUM (Willd.) DC., Cat. Hort. Monspel. 149. 1813; *Leontodon laevigatus* Willd., Sp. Pl. **3**:1546. 1800; *T. erythrospermum* Andrz. in Besser, Enum. Pl. Volhyn., Podol., etc., 75. 1822; *L. erythrospermum* Eichw., Naturhist. Skizze Litth., Volhyn, etc., 150. 1830; *L. erythrospermum* Britton in Britt. and Brown, Ill. Fl. N. Amer. ed. 2., **3**:316. *fig. 4064*. 1913; *T. mexicanum* Wooton and Standley, Fl. New Mex. 626. 1915 (non DC.).

Herba subgracilis, 5–30 cm. alta. Radix tenuiuscula, simplex vel pluriceps, fusce corticata, collo foliorum vetustorum fragmentis persistentibus magnus, plerumque pallide brunneis large squamato, longe lanuginoso vel rarius glabro. Folia terrae adpressa vel suberecta, glabra vel infra parce pilosula, lanceolata 0.5–4 (vel serius etiam −11) cm. lata, versus basim plerumque longe angustata, fere semper tota profunde incisa vel variis modis usque ad nervum medium crebre pinnatisecta, lobis latis angustisve, plerumque acutis, integris vel largius et tenuiter dentatis, plus minusve reflexis, interiectis saepe lobulis dentiformibus, lobo terminali lateralibus paulo maiore vel etiam interdum minore. Scapi singuli vel numerosi, subtenues, erecti vel e basi procumbente adscendentes, floriferi foliis breviores vel longiores, serius plus

[26] "Nimis *T. Denti-leonis affine et forte varietas*," DC., *loc. cit.*

minusve elongati. Capitula parva vel mediocria, circum 1–2 cm. longa et paulo latiora. Involucri foliola utriusque seriei circa 11–13, pallidius atriusve griseo-viridia, glauco-pruinosa, ecorniculata vel plerumque corniculis mediocribus vel parvis instructa. Exterioris seriei foliola adpressa, patentia vel e basi patenti recurva, interioribus latiora eorumque longitudinis tertiam vel dimidiam partem vix superantia, infima ceteris breviora, omnia late vel angustius ovata vel e basi ovata triangularia (4–8 mm. longa et 1.5–3 mm. lata), margine membranaceo plerumque distinctissimo. Flores numerosi, involucro 2–4 mm. longiores, citrini vel (in speciminibus pinguibus) *T. vulgaris* floribus paulo tantum pallidiores, extus plerumque griseo vel rubro striati. Achaenia parva, ± 3–4 mm. longa, intense rufa, rufopurpurea vel fere atropurpurea, supra tuberculis longis angustis largis obsita, saepe infra quoque rugulosa, in cuspidem anguste linearem longam, totius fructus tertiam vel quartam partem superantem abrupte contracta. Rostrum tenue, achaenio dimidio vel plus duplo longius. Pappus albus, 4–7 mm. longus.

DISTRIBUTION.—Nova Scotia and Virginia to British Columbia, Idaho, and New Mexico; apparently introduced from Europe, where native, as also (fide Handel-Mazz.) in western Asia and northwestern Africa.

SPECIMENS EXAMINED.—Nova Scotia: Yarmouth, *John Macoun*, June 3, 1910 (Hb. Can. 81358).

Massachusetts: Dorchester, May 24, 1903, *John Murdoch, Jr.*, 1304 (Hb. Field 470218); Weston, May 21, 1904, *idem* 1625 (Hb. Field 470265).

Vermont: Vergennes, dry knolls in orchard, May, 28, 1899, *Ezra Brainerd* (Hb. Greene 48444); West Rutland, Twin Mountains, May 31, 1899, *W. W. Eggleston* 1416 (Hb. U.S. 364398); Shrewsbury, May 25, 1902, *idem* 2681 (Hb. U.S. 492337).

Pennsylvania: Rohrerstown, April 21, 1891, *John K. Small* (2 sheets in Hb. Field, 169751 and 169752); vicinity of Conewago, May 14, 1891, *idem* (Hb. Field 167811).

District of Columbia: Washington, university grounds, May 2, 1899, *Edward L. Greene* (Hb. Greene 48443).

Virginia: North Four Mile Run, May 1898, *Ivar Tidestrom* (Hb. Greene 48441).

Michigan: Durand, May 15, 1913, *Edward L. Greene* (Hb. Greene 22276).

Ohio: Sandusky, July 28, 1903, *E. L. Moseley* (Hb. Field 240265).

Illinois: Chicago, in yard, May 1919, *Winnifred Baantjer* (Hb. Field 485106); Urbana, University campus, April 1, 1907, *Frank C. Gates* "1369.3"

(Hb. U.S. 648981); White Heath (Piatt Co.), ballast of railroad, May 4, 1907, *idem* 1432 (Hb. U.S. 649007); Evanston, near walk, July 4, 1919, *Earl E. Sherff* 3089 (Hb. Field 484466 and 484467).

Missouri: Vicinity of Springfield, pastures, August 28, 1911, *Paul C. Standley* 8287 (Hb. U.S. 687249).

Nebraska: Omaha and vicinity, street, August 16, 1905, *Amy C. Lawton* 65 (Hb. Field 193610).

New Mexico: Chama (Rio Arriba Co.), alt. 2380 m., May 26, 1911, *W. W. Eggleston* 6665 (Hb. U.S. 660876).

Alberta: Athabasca Landing, July 28, 1914, *A. S. Hitchcock* 12158 (Hb. U.S. 885176).

Idaho: Coeur d'Aleur, abundant in lawns at city limits, August 11, 1913, *Henry J. Rust* 396 (Hb. U.S. 870324).

Wyoming: Yellowstone National Park, June 4, 1902, *Edgar A. Mearns* 939 (Hb. U.S. 486330).

British Columbia: Beavermouth, floodplain of Columbia, alt. 2400 ft., August 18, 1905, *C. H. Shaw* 1149 (Hb. U.S. 622044).

This species should not be confused with *T. laevigatum* A. Gray (Proc. Acad. Phil. 1863:70), which was synonymous with *T. lyratum* (Led.) DC. In recent American literature it has been known as *T. erythrospermum*, but HANDEL-MAZZETTI (Monogr. Taraxacum 109. 1907) has seen WILLDENOW'S original specimen of *Leontodon laevigatum* and found that *T. erythrospermum* is purely synonymous with it. BRITTON (*loc. cit.*), familiar only with the name *Taraxacum erythrospermum*, but rejecting the generic name *Taraxacum*, has lately used the name *Leontodon erythrospermum* for this species; but this last combination (made by EICHWALD in 1830) is untenable of course, since under the appellation *Leontodon*, the name *Leontodon laevigatus* antedates it by a number of years. WOOTON and STANDLEY (*loc. cit.*) have confused this species with *T. mexicanum* DC. (*T. vulgare*). From their herbarium determinations and also from their description, "achenes red," it is seen that their plants were purely *T. laevigatum*.

Specifically, *T. laevigatum* is much the most clearly marked and sharply defined of any of our native or introduced North American species of *Taraxacum*.

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SHERFF on TARAXACUM

SHERFF on TARAXACUM

SHERFF on TARAXACUM

EXPLANATION OF PLATES XXXI–XXXIII

PLATE XXXI

Taraxacum lyrata

FIGS. *a* and *j* (×0.48). *T. alaskanum* form: *Coville* and *Kearney* 1097, Haenke Isl., Hb. U.S. 376702; figs. *b* and *f* (×0.48), foliage form typical as to LEDEBOUR'S type illustration, but plants less compound at base than figured by LEDEBOUR, *Coville* and *Kearney* 2164, St. Matthew Isl., Hb. U.S. 376718; figs. *c*, *g*, and *i* (×0.63), showing (*c*) foliage form of *T. lyratum* that corresponds to *T. mutilum* form of *T. ceratophorum* and matches type figures of *T. hyperboreum* Dahlst. and *T. eurylepium* Dahlst., (*g*) foliage form matching VAHL'S type plate of *T. phymatocarpum*, and (*i*) foliage form of *T. lyratum* more nearly approaching that of LEDEBOUR'S plate, all three from *Walpole* 1791, Alaska, Hb. U.S. 378905; fig. *d* (×0.56), from type sheet of *T. alaskanum*, McIlhenny 111, Alaska, Hb. N.Y.; fig. *e* (×0.52), topotype of *T. alaskanum*, Murdoch, Alaska, Hb. U.S. 424063; fig. *h* (×0.70), foliage form closely matching some of more erect "*T. phymatocarpum*" forms from Greenland, *Knowlton* 142, Arizona, Hb. U.S. 41949; fig. *k* (×0.79), tiny dwarf form of Rocky Mts. (*T. officinale* var. *scopulorum* Gray), *Baker*, *Earle*, and *Tracy* 293, Colorado, Hb. U.S. 76097; fig. *l* (×0.67), from type sheet of *T. rupestre*, *Macoun*, British Columbia, Hb. Can. 15111.

PLATE XXXII

Taraxacum ceratophorum

FIG. *a* (×0.47),—Type material of *T. mutilum*, *Glenn*, Alaska, Hb. U.S. 376755; fig. *b* (×0.36), from type sheet of *T. lacerum*, *Dawson*, northern British-Columbia boundary, Hb. Can. 15119; fig. *c* (×0.44), type plant of *T. leiospermum; Osterhout* 2645, Colorado, Hb. N.Y.; fig. *d* (×0.47), authentic material of *T. Chamissonis*, Cole, St. Paul Isl., Hb. U.S. 376691; fig. *e* (×0.37), from one of type sheets of *T. dumetorum*, *Greene*, Wyoming, Hb. Green 48431.

PLATE XXXIII

Taraxacum eriophorum

FIG. *a* (×0.64).—From type sheet of *T. eriophorum*, *Mrs. Fitch*, Montana, Hb. Mont. Agric. Exper. Station; fig. *b* (×0.52) from one of type sheets of *T. angustifolium*, *Greene*, Wyoming, Hb. Greene 48451; fig. *c* (×0.59), from type sheet of *T. ovinum*, *Macoun*, Alberta, Hb. Can. 11711; fig. *d* (×0.47), from type sheet of *T. ammophilum*, *Nelson*, Wyoming, Hb. Greene 48427.

VISCOSITY VALUES OF PROTOPLASM AS DETERMINED BY MICRODISSECTION[1]

WILLIAM SEIFRIZ

Introduction

The first descriptions of protoplasm, written nearly a century ago, characterize it as "a living jelly." While protoplasm is often of high viscosity, any restricted statement is likely to be misleading, for the viscosity of protoplasm may vary from a consistency slightly more than that of water to that of a firm jelly. From descriptions to be found in current literature it is rather difficult to know of just what degree of viscosity the living substance might be. The difficulty lies in the fact that there has been no careful systematic attempt to ascertain the exact degree of consistency of protoplasm from numerous types of cells and under many different physiological conditions. The following paper represents such an attempt.

Method

The method used has been that of microdissection. The instrument employed in this method is a modification of the Barber pipette holder (1). It consists essentially of two needle holders, each capable of three movements. The holders are fastened to the microscope stage, and the two needles held in them project into a glass moist chamber in which the material to be worked upon is suspended in a hanging drop of water on the under side of a cover slip which forms the roof of the chamber. The needles are of glass and possess exceedingly fine but rigid tips. The technique of the microdissection method is fully described by CHAMBERS (7), to whose article the reader is referred.

The harmful consequences which are likely to result from the practice of holding material in a thin water film, and the importance of this to microdissectionists, make it advisable to direct attention

[1] Botanical Contribution from the Johns Hopkins University, no. 66.

to the frequently damaging effect of this method by presenting some experimental data.

In working with material in a hanging drop it is often very desirable to have the water film of such thinness that the material is held firmly against the cover slip. The surface tension between water and glass thus produced is quite sufficient to hold an active protozoan such as *Euplotes* in a fixed position, or to flatten out marine ova, making them more transparent and dissection easier. If the material thus subjected to surface tension suffers no appreciable distortion, no harmful consequences may result, but eggs of seaweeds and echinoderms are sufficiently pliable to be readily distorted, so that the protoplasm is subjected to an abnormal strain which frequently causes rapid deterioration.

In order to ascertain in a general way the harm done through the flattening of marine ova by a water film, I made a series of observations on the ova of the sea-urchin *Tripneustes*. These eggs were placed in a large and much spread water droplet, which was of greater depth at its center than the diameter of an egg, while toward the periphery the water film gradually thinned to an imperceptible depth. Ova in the center of the droplet were of a normal spherical shape, while those in the peripheral film were much flattened. In half an hour the viscosity of the protoplasm of those eggs in deep water had risen only slightly, while the viscosity of those in the peripheral film had greatly increased. In an hour the spherical ova had risen in viscosity a barely perceptible amount, while the flattened ones had become a firm gel. Furthermore, the distorted eggs tolerated less dissection before showing pronounced disorganization, retained the capacity for healing a wound for a shorter time, and, in a great number of cases, their entire contents dispersed suddenly at the first touch of a needle.

While the use of the surface tension of a thin water film is of value in holding material for microdissection, therefore, the method must be used cautiously. Indeed, the greatest care possible must be taken to keep the material living and normal, and to become familiar with those criteria which assist in ascertaining the exact condition of the living substance. A full appreciation of the extreme irritability of protoplasm is a prerequisite to successful microdissection.

Terminology

Biological terms of uncertain connotation will be considered when the term is first used. I shall discuss briefly a few important words in colloid chemical nomenclature which have no fixed and precise meaning to either chemist or biologist, although they are extensively used by both. THOMAS (**26**) has recently published an account of the nomenclature employed in colloid chemistry, and has in most instances suggested the preferable term to use. I shall follow his terminology strictly, with the possible exception of the words "gel," "jelly," and "coagulum," regarding which THOMAS comes to no definite decision.

In view of the widespread and lax use of the term gel it seems advisable to use the word for the general condition of a colloidal sol when it hardens, whether the hardening be through pectization, that is, coagulation of the sol (the original sense in which gel was used by GRAHAM), or whether through setting (of a gelatine sol, for example), that is, gel includes both the stiffened jelly-like form of a sol, which will redisperse, and the hard amorphous form which will not redisperse. I shall designate gels by stiffening as jellies (which has the support of BECHHOLD **3,** p. 4), and gels by coagulation as coagula.[2] Thus do we also have the corresponding verbs gelate (proposed by TAYLOR **25,** p. 10), to form gels; set, to form jellies; and coagulate, to form coagula.[3]

The employment of three terms where two might suffice may be objectionable in some respects, but it has much to recommend it when protoplasm is the colloid being studied. When protoplasm stiffens it is impossible always to determine definitely whether the mass is a coagulum or a jelly. If the hardening is excessive an undoubted coagulum results, for the living substance is then irreversible, and can be cut up into solid chunks, while, if the

[2] THOMAS (**26,** p. 12) states that BECHHOLD restricts "gel" to the coagula of sols, and terms stiffened sols "jellies." The former statement is not supported by BECHHOLD'S more recent work (**3,** p. 4) where he says: "It seems preferable to me to use the expression "gel" for the general comprehensive phenomenon, and to reserve the word "jelly" for the gelatinization of a hydrophile colloid" (that is, for the gelatinized hydrophile colloid).

[3] HARDY (**12**) employs the last two terms in the same way. "The production of an insoluble gel I shall call "coagulation," that of a soluble gel "stiffening."

increase in consistency is not too great, a firm but resilient and cohesive jelly results. Between these two conditions the exact colloidal state cannot be readily determined. The general term gel is consequently applicable.[4]

Scale of viscosity values

All sciences suffer from a confusion in nomenclature, but perhaps biology more than any other. For example, "viscosity" to the physicist is a specific and accurately measurable property of matter. All fluid substances have their mathematically determined coefficients of viscosity in physics, but to the biologist viscosity has a meaning no more definite than that the substance described has a density somewhere between that of water and of steel. This vagueness, however, is not altogether the biologist's fault.

Protoplasm cannot be collected in sufficient quantity, nor would it remain normal long enough to determine its viscosity, as the physicist would determine that of a liquid, by running it through a viscosimeter; consequently, any attempt to specify the consistency of protoplasm will be more or less influenced by personal opinion. The personal element, however, can be restricted to observations, and not be allowed also to determine the connotation of loose expressions, such as "non-viscous" (an impossible term) and "very viscid sol," which may have quite a different meaning from that conveyed to the reader.

In a previous paper (23) a scale of viscosity was devised so that an expression such as "decidedly viscous" would hold a definite place, and thus convey some idea of approximately how viscous a "decidedly viscous" substance is. I shall retain this

[4] I have heard from the younger colloid chemists that true gels are obtainable only from emulsoids; therefore the coagulation of a suspensoid is not a gel; that is, a gel is not a coagulum; it is a jelly. Some gels (jellies) are reversible and some not. To exclude coagulation as a word descriptive of the process of hardening of emulsions would be altogether too radical a departure in biology, where we constantly speak of the coagulation of blood, of albumin, and like emulsions. I shall, with BECHHOLD, consider as coagulations such processes as cause an irreversible change, whether from emulsions or suspensoids. It would be a great service to biologists, and undoubtedly to colloid chemists themselves, if the usage of gel, jelly, and coagulum were fixed, even though arbitrarily and but tentatively.

scale, but give in addition viscosity values to each degree of consistency. These values are directly referable to definite concentrations of a dispersion (colloidal solution) of gelatine, each concentration, as nearly as could be determined, having the same consistency as that of the protoplasm which has been given a corresponding viscosity value. The gelatine used is a commercial one and readily obtained.[5]

Examination of the gelatine dispersions was done at room temperature (18° C.) in the moist chamber of the microdissection instrument, with needles identical with those employed for the dissection of protoplasm. Table I shows the viscosity values and

TABLE I

Viscosity value*	Descriptive term	Percentage of gelatine†	Substances having an equivalent viscosity‡
1.....	Watery	0.0	Water
2.....	Very liquid	0.05	.....
3.....	Liquid	0.2	.....
4.....	Slightly viscous	0.4	.....
5.....	Rather viscous	0.5	Paraffine oil.....
6.....	Decidedly viscous	0.6	.....
7.....	Very viscous	0.7	Glycerine
8.....	Extremely viscous	0.8	Bread-dough
9.....	Gel	1.0	Vaseline
10.....	Rigid gel	2.0	Firm gelatine

*The abbreviation v.v. will be used for viscosity value.
† A 1 per cent dispersion is a mixture of 1 gm. of gelatine in 99 cc. of water.
‡ All substances were examined at room temperature, 18° C.

corresponding percentages of gelatine which have been established, and these will serve as standards for the viscosity values of protoplasm given in this paper.

Just how accurate percentages of gelatine dispersions are going to prove to be as standards of viscosity is somewhat doubtful, in view of the sensitivity of gelatine to such influences as electrolytes, length of period warmed (**25,** p. 121), etc., which greatly influence its solidification temperature. The percentages of concentration at which gelatine will set, as given in the literature, vary considerably. TAYLOR states that a 10 per cent dispersion of gelatine

[5] Bacto-gelatine, "Difco" standardized, in granular form, with a moisture content less than 4 per cent, made by the Digestive Ferments Company of Detroit, and sold by Arthur H. Thomas Company of Philadelphia.

will set at a temperature of 21° C. This value as a minimum for that temperature must certainly be erroneous. BECHHOLD speaks of a 1 per cent dispersion of gelatine as a semifluid gel (presumably at room temperature), and states that "aqueous" solutions (containing only 1 per cent of water-free gelatine) gelatinize at "ice-box temperature." I find that as low as an 0.8 per cent dispersion (depending on the kind of gelatine used) will gelatinize, that is form a soft jelly, at 18° C. In view of the fact that I have employed a standardized gelatine, it is possible that the values of viscosity given may be fairly accurately verified. I have deemed it advisable to add another column of familiar substances, however, which will help stabilize the scale, and which will also more clearly indicate the exact consistency meant by any given value.

Viscosity as a criterion of the sol and gel states

While a high consistency of an emulsion is almost conclusive evidence of the gel state and a very low consistency evidence of the sol state, yet viscosity alone is not a dependable criterion of the sol and gel conditions. When the viscosity is of an intermediate grade it is of no value as even an approximate indication of the colloidal state. It will be well, therefore, to consider to what extent we are justified in using the terms gel and sol as descriptive of the physical state of protoplasm when viscosity is the only criterion. Although we read that "gels are solid" (**19,** p. 230), and although the word gel connotes to most of us a rather firm though elastic body, it is not necessarily true that all gels are rigid. In fact, this is not the case; a gel may be quite soft. FREUNDLICH, I believe, uses as an indication of the gel state the fact that the substance will support a glass rod placed upright in it. Any criterion of the gel state based on viscosity alone at best can be only approximate. Physical structure and not viscosity determines the colloidal state. We have really no ready means of determining in all instances the actual physical structure of protoplasm, and probably, therefore, should do away with the expressions sol and gel when the degree of viscosity is the only indicator of one state or the other. Where the consistency is very high or very low, however, we can safely characterize the living emulsion

as being respectively in the gel or sol state. It is the intermediate degrees of viscosity of protoplasm of whose physical structure and therefore colloidal state we are totally ignorant. Extremely viscous protoplasm (v.v. 8) is probably a gel, but whether very viscous or decidedly viscous protoplasm is cannot be conclusively stated. This matter may not appear to be a serious one at first, but a brief consideration of the indiscriminate use of the term gel in the literature will show the misinterpretations to which such usage leads.

BAYLISS (2) states: "There is one fact about which there can be no doubt, that is, that protoplasm behaves as a liquid"; while MATHEWS (19) states that protoplasm "is a jelly-like substance or technically a gel." An older investigator (RHUMBLER 22), during a discussion on the structure of protoplasm, says that "protoplasm cannot be a solid substance," while a recent worker (HYMAN 13) says, the fact "that isolated pieces of protoplasm assume the spherical form is not necessarily a proof of its fluid condition," and that "protoplasm is in the gel state." Each of these statements is supported by some experimental facts, but each is true only in part. Protoplasm does not always behave as a liquid (the highly viscous, quiescent protoplasm of bread mold, for example), nor is it always a gel (as for example the very liquid endoplasm of *Euplotes*). KITE (15) has well expressed the exact state of affairs as follows: "Living matter occupies an intermediate position between true solids and true liquids and has many of the properties of both as well as properties peculiar to itself. It belongs to the class of colloids known as emulsoids and exists in either a gel (hydrogel) or a sol (hydrosol) state." Unfortunately, however, KITE then proceeds to use gel as descriptive of protoplasm regardless of viscosity. To be sure, viscosity is not a precise index of physical structure, but with ordinary illumination we have no other criterion by which to judge the exact colloidal state of protoplasm, and a low viscosity is strongly suggestive of a sol, or at least does not suggest a gel state. Such statements as "The living endoplasmic substance is a very dilute and apparently homogeneous gel," and "This structure (the jelly surrounding the egg of *Asterias*) has a low viscosity for a gel and is therefore extremely dilute," are inexact and can

only lead to misinterpretations regarding the true viscosity of protoplasm.

It is very probable that much of the unfavorable criticism of KITE's work is due, not to faulty observations, but to his lax use of the term gel. For example, GARREY (10) claims that the perivitelline space of a fertilized echinoderm egg is filled with a liquid and not with a gel as KITE (according to GARREY) maintains; but who is to decide that the liquid which GARREY sees is not the same kind of substance which KITE saw if gel means one thing to KITE and another thing to GARREY? Sol and gel should be used with great caution in describing the physical state of matter when one is dealing with an emulsion. It is better to give some idea of the viscosity of the substance and let that suggest a possible sol or gel state. It is well to emphasize the fact that protoplasm is not a simple two-phase colloidal system, as one is led to believe from reading much of the literature. On the contrary, it is a multiphase system, emulsion within emulsion.

General viscosity values of protoplasm

The viscosity of protoplasm is not fixed, for it varies in different organisms, in the same organism at different times, and even in different regions of the same organism at the same time. Furthermore, the viscosity of the constituent parts of the protoplasm (the matrix and the protoplasmic inclusions) may differ from the viscosity of the protoplasm as a whole, and from each other. The examination of an inactive Myxomycete plasmodium will frequently reveal a ground substance of very low viscosity, while the mass of protoplasm as a whole is of high consistency. This relation between the viscosity of the constituents of an emulsion and that of the emulsion as a whole is very evident in certain artificial emulsions. For example, in a dispersion of gelatine the viscosity of the medium (water) at 20° C. is 0.012, while the viscosity of the emulsion, of only 2 per cent concentration, is three times as great (0.037).[6] The most striking example of the high consistency of an emulsion as compared with the low viscosity of its dispersion

[6] This reading (from TAYLOR 27) seems surprisingly low, since a 2 per cent concentration of gelatine will ordinarily set into a jelly at a room temperature of 18° C.

medium is the case of castor-oil soap, a 0.1 per cent concentration of which is an almost solid jelly (21).

Thus is it seen that in giving viscosity values it is necessary to distinguish between protoplasm as a whole and its constituent parts, especially the matrix (hyaloplasm). I prefer to use the term matrix rather than hyaloplasm, owing to the confusion which exists in the use of the latter word. Hyaloplasm, as first used by HANSTEIN (11), designated "the homogeneous ground substance" of protoplasm as distinguished from the granules suspended in it. Homogeneity of the ground substance, however, is not definitely established. CHAMBERS (6) and WILSON (27) have employed hyaloplasm to mean the "interalveolar substance." WILSON (29), however, admits the possibility of using the word in the exactly opposite connotation; that is, the ground substance (also termed cell sap, enchylema, hyaloplasm, etc.) is the "alveolar substance" which fills the alveoli. If we accept BÜTSCHLI'S (4) contention that the hyaloplasm (the peripheral granular-free border) of Myxomycetes is not homogeneous, but is of a definite alveolar structure, then this hyaloplasm must be regarded as including both phases of the emulsoid structure; that is, as consisting of interalveolar and intraalveolar substance.

Material

The data upon which the following discussions are based were obtained by a study of a considerable variety of material. Consequently, the conclusions reached may be regarded as rather generally applicable. Since prominent dissimilarities do occur in the properties of widely differing and sometimes of closely related genera, however, it is to be understood that the statements made refer only to the organism under discussion at the time, although many of the general deductions apply to the protoplasm of all the organisms worked upon, if indeed they are not applicable to all living substance.

The following types are the chief ones which were used for this study: the Myxomycetes *Ceratiomyxa*, *Badhamia*, *Arcyria*, *Cribraria*, and *Fuligo;* the rockweed *Fucus;* the fresh water algae *Spirogyra* and *Vaucheria;* the bread molds *Rhizopus* and *Zygorhynchus;* pollen tubes of the blue-flag *Iris versicolor*, of the beach-

pea *Lathyrus maritimus*, and of the dog's-tooth violet *Erythronium revolutum;* the protozoa *Amoeba* and *Euplotes;* the sand-dollar *Echinarachnius;* and the sea-urchin *Tripneustes esculentus*.

The experimental work on these forms was done mostly in the Botanical Laboratory of the Johns Hopkins University. The work on *Fucus*, *Echinarachnius*, and part of that on Myxomycetes and pollen tubes was carried on at the Harpswell Laboratory, South Harpswell, Maine.[7] The work on *Tripneustes* was done at Ocho Rios, Jamaica, B.W.I.[8]

I am greatly indebted to Professor DUNCAN S. JOHNSON for first pointing out to me the possibilities of microdissection as applied to the study of living protoplasm, and for assistance during the progress of this work. To Professor ROBERT CHAMBERS of the Cornell Medical College, New York City, my thanks are due for many suggestions relative to microdissection. I wish also to acknowledge the help received from Dr. CHARLES V. TAYLOR pertaining to the structure and behavior of the protozoan *Euplotes*, and from Dr. HOWARD E. PULLING, then of this university, pertaining to problems in physical chemistry. To Professor WARREN K. LEWIS of the Medical School of this university I am indebted for the loan of the microdissection instrument used in this work.

Myxomycetes

The consistency of Myxomycete protoplasm when in the active vegetative state is liquid (v.v. 3). One is quite likely to be misled by the apparent ease with which a needle traverses protoplasm into believing that the protoplasm is of watery consistency. Superficial observation of streaming protoplasm also leads one to believe that it must be very liquid, while, as a matter of fact, it may be rather viscous, as in bread mold. A good indicator of the degree of viscosity, when the protoplasm is of low consistency, is the distance from the path of a moving needle at which granules are disturbed. The presence of Brownian movement suggests a low consistency.

[7] I am indebted to Director J. S. KINGSLEY for the use of a room at the Harpswell Laboratory.

[8] To FRANK CUNDALL I am greatly obliged for his kindness in placing the facilities of the Institute of Jamaica at my disposal.

The density of the quiescent plasmodium is very high, possessing a maximum viscosity value of 8, and at times approaching the consistency of a gel, but not possessing the firmness of a rigid gel, for the protoplasmic mass of a quiescent Myxomycete is poorly resilient, although quite tough and elastic, and often possessed of a somewhat plastic quality, in this respect closely resembling bread dough. One prominent characteristic of Myxomycete protoplasm is that it is extremely glutinous. This is in striking contrast with marine ova, the protoplasm of which is not noticeably mucilaginous. As a Myxomycete prepares to fruit, the protoplasm increases in viscosity, until it becomes of gel consistency.

Very frequently a tear in a highly viscous, inert plasmodial mass will cause the formation of a rapidly enlarging protrusion. The liquid which flows into and increases the size of such a globule is a granule-free, hyaline substance, to all appearances identical with the peripheral hyaloplasm, but its origin is not peripheral, for this flow of translucent fluid has its source within the protoplasmic mass. Such behavior seems to favor LEYDIG'S conception of the structure of protoplasm, namely, a framework of spongioplasm permeated by a more liquid hyaloplasm (enchylema). By the use of pressure REINKE and RODEWALD (4) obtained 66 per cent of fluid enchylema from the plasmodium of *Aethalium*. The nature of this exuded liquid substance from a plasmodium cannot be stated with certainty. It appears to be the matrix in which the protoplasmic granules are imbedded, or, more accurately, the enchylema (interstitial substance), since the protoplasm is in the gel state and probably of sponge structure.

Amoeba

In many respects *Amoeba* closely resembles the slime molds. Both organisms have periods of motility and non-motility. The former period is characterized by protoplasmic streaming and the formation of pseudopodia, and by a rather liquid condition of the protoplasm; the latter, by protoplasm that is quiescent and more viscous. Both types of organisms are also differentiated into three more or less distinct regions, namely, the inner less viscous

endoplasm, the outer more viscous ectoplasm, and the peripheral highly viscous protoplasmic membrane.[9]

The flowing endosarc of an active *Amoeba* is of slightly viscous consistency (v.v. 4). When quiescent, the endoplasm becomes of a rather or even a decidedly viscous density (v.v. 5 or 6), but seldom higher, never in the living condition attaining a gel consistency (encystment would probably be an exception to this). Brownian movement of particles is generally present and very pronounced throughout the endoplasm of an active *Amoeba*. This suggests a liquid condition. In the quiescent protoplasm of an inactive *Amoeba* the number of particles exhibiting Brownian movement is decidedly less and the amplitude of vibration is reduced, which are evidences of an increased viscosity. The viscosity of the ectosarc is much higher than that of the endosarc, and, as in the latter, varies inversely with activity. The most pronounced decrease in consistency of the ectoplasm occurs in the region immediately concerned in pseudopodium formation, that is, at the tip of an advancing pseudopodium. Here, in a rather restricted center, the ectoplasm becomes quite liquid, which condition, of course, is conducive to the making of a pseudopodium. The liquid condition is temporary and brief. The ectoplasm not directly taking part in amoeboid movement is of very viscous consistency (v.v. 8).

Investigators generally recognize the high viscosity of the ectosarc of *Amoeba*. For example, JENNINGS (14) says that the ectosarc shows "the characteristics of matter in the solid state of aggregation," and HYMAN (13) concludes that the ectoplasm is a gel, "semi-rigid and more or less solidified." The latter, however, although recognizing the possibility of "real fluidity" of the "surface layer," goes too far when assuming that the ectoplasm may attain "extreme solidity." This conclusion is apparently based in part on KITE's (15) statement that "little difficulty is experienced in cutting it (the ectoplasm of *Proteus*) into pieces as small as the limit of microscopical visibility." Here (as in the

[9] Some observers would restrict this differentiation to two regions, not recognizing a distinct protoplasmic membrane.

case of the cytoplasm of the *Asterias* egg which KITE describes as "a quiet translucent gel . . . . which can be cut into small pieces") KITE was either dealing with dead protoplasm, or else the expressions used convey an impression which he himself did not mean. In none of the material worked upon have I been able to "cut" the living protoplasm into small pieces.

There is no doubt that when one is able to cut protoplasm "into pieces as small as the limit of microscopical visibility," the protoplasm is no longer normal. It is not clear just what degree of viscosity KITE wishes to attribute to the ectosarc of *Amoeba*. He states that "this living substance has a moderately high viscosity," but he also says that it is a "quite concentrated gel" (p. 155). KITE'S use of the term gel is very broad. The nearest approach to a firm gel condition of the protoplasm which I have studied is that of the ectosarc of the ciliate *Euplotes* and the quiescent protoplasm of bread mold, but even here the protoplasm possesses considerable plasticity, and, although holding its shape when freed, is of soft rather than solid consistency.

HYMAN credits CHAMBERS with confirming these results of KITE. This is not altogether true. To be sure, CHAMBERS (5) does say that "the external surface of the egg is a gel," and that the surface layer of marine ova is directly comparable with the "rigid ectoplasm" of Protozoa; but he nowhere states that normal living protoplasm can be cut into small pieces. Quite the contrary, he calls attention to the fact that it is the protoplasmic "coagulate" (and a coagulum, as HYMAN points out, is "incompatible with life") that can be "cut into pieces which hold their shape"; and adds that this is likely "to lead one to the erroneous conclusion that the substance of a cell is usually a solid protoplasmic gel." It is true, however, that the ectoplasm of *Amoeba*, when not immediately concerned in pseudopodium formation, is of high consistency, possessing, as HYMAN says, many properties of solids, such as great elasticity, extreme viscosity, and compressibility; and this is sufficient to support the interesting theory (first advanced by MONTGOMERY, according to MCCLENDON 20) that amoeboid movement is "due to alterations of the colloidal state" (13), that is, it is a solation-gelation phenomenon.

Rhizopus

In the bread mold *Rhizopus* (and *Zygorhynchus*) we have, as in Myxomycetes and *Amoeba*, two general states of consistency, changing from one to the other with changes in physiological activity. The protoplasm in the hyphae of bread mold, in the quiescent state, is of very high consistency. It possesses the greatest viscosity of any living plant protoplasm which I have observed by the aid of microdissection.[10] It is of gel consistency, more usually that of a soft gel (v.v. 9), is sticky, quite elastic, and very extensile, closely resembling bread dough in these physical properties. At times it exhibits some resiliency, and may then be characterized as a rigid gel (v.v. 10). KITE (**15**) has described the living substance of the striped muscle cell of *Necturus* as "the most viscous, elastic, and cohesive of the living gels we have so far considered." The protoplasm of the cells of nerve and muscle tissue is probably of as high a viscosity as any living animal plasma; but it must not be concluded from these observations that this gel consistency is necessarily permanent. Nerve and muscle protoplasm probably exist, just as all the protoplasm so far considered exists, in the sol state at times. Indeed, certain theories of muscle contraction based on "a temporary redistribution of the more fluid portion of the tissue" (LILLIE, **18**), and on "coalescence (incipient coagulation) of colloidal particles," which is reversed during the relaxation phase (**17**), demand a varying viscosity of muscle cells.

Ordinarily, when a filament of bread mold is torn the inactive protoplasm will not flow out, but when pressure is exerted by a needle some distance back from the torn end, the gelled protoplasm can be forced out in the form of a rod, just as one would squeeze oil paint from an artist's tube, and this rod maintains its shape until disturbed.

The streaming protoplasm of *Rhizopus*, as one would expect, is considerably less viscous than the quiescent protoplasm, but it

[10] The viscosity of plant protoplasm which is undergoing long periods of rest, for example, that of seeds, is undoubtedly of even greater concentration, for here the water content is reduced to 20 per cent, while in protoplasm in which pronounced metabolic processes are going on the percentage of water is 80 or more.

is not of as low viscosity as it superficially appears to be, and by no means closely approaches the very liquid state of the endoplasm of an active *Amoeba*. In its most fluid condition it is of at least a rather viscous consistency (v.v. 5), while, when slowly streaming, it may be of very viscous consistency (v.v. 7). All gradations exist between the rather viscous condition when streaming and the gel state when quiescent.

Increase and decrease in viscosity of protoplasm in *Rhizopus* are probably dehydration and hydration phenomena. It is interesting to appreciate the extreme rapidity with which these changes may take place. The streaming protoplasm, by pressure of a needle sufficient to close a hypha, may be made to assume instantly such a consistency that not only does streaming cease, but on subsequent tearing of the filament the emptying of the thread is prevented. Choking of the hypha has caused gelation of the plasma, probably through dehydration. Later, without further disturbance by needles, there is a reversal of the phenomenon. Solation takes place (apparently hydration has set in) and the protoplasm of itself flows out of the torn filament.

Euplotes

In the ciliate *Euplotes* there exists a differentiation between endoplasm and ectoplasm more marked than in any other instance of which I am aware. The endoplasm is very liquid, while the ectoplasm has the firmness of a rigid gel. The former consists of a dilute matrix in which a great variety of inclusions are suspended, from minute protoplasmic particles to whole Protozoa taken in as food; while the latter is free of minute granules (microsomes), and presents a beautiful alveolar structure with the characteristic surface alveolar layer. So far as my limited observations on this protozoan go, the very liquid condition of the endoplasm and the gel state of the ectoplasm seem to be constant. I have observed no change from sol to gel and vice versa, nor any appreciable increase or decrease in viscosity. That there must be some such change at division of the organism (for example, solation of the rigid ectoplasm) seems a physical necessity.

Marine ova

The fully mature, normally discharged eggs of *Fucus* are decidedly viscous (v.v. 6). The unripe eggs are of lower viscosity. The properties and behavior of the ova of *Echinarachnius* and *Tripneustes* are so similar that they can be considered together. The consistency of the mature unfertilized eggs of *Echinarachnius* and *Tripneustes* is a trifle higher than the protoplasm of *Fucus* ova, but barely of very viscous consistency (v.v. 7). KITE has described the cytoplasm of the *Asterias* egg as "a quiet translucent gel." CHAMBERS (5) calls attention to the fact that KITE's paper "is a pioneer one in microdissection research. The observations recorded were necessarily fragmentary." KITE was probably dealing with degenerate gelated protoplasm, or else he fully appreciated the true viscosity of the normal protoplasm of an echinoderm egg and has erroneously described it by his loose use of the term gel. CHAMBERS (6) states that "the interior cytoplasm of a marine egg is a viscous fluid. The viscosity is high enough to prevent any Brownian movement of the inclosed granules." Since the expression "viscous fluid" holds no place in a scale of viscosity, nor is it compared with any other commonly known substance, it is not quite clear just how viscous is the living viscous fluid of a marine egg. The minimum viscosity which the absence of Brownian movement (a criterion used by CHAMBERS) will permit is apparently the viscosity of concentrated laboratory glycerine. EXNER, according to LEHMAN (16), found that "the concentration of ordinary commercial glycerine (specific gravity 1.21) was just enough to put a complete stop to the vibration." A very slight dilution of concentrated glycerine (specific gravity 1.25) is sufficient to permit a noticeable Brownian movement of suspended carmine particles. If Brownian movement is impossible in a very viscous substance such as glycerine, which has a viscosity value of 7, then protoplasm in which no Brownian movement is evident must apparently possess a consistency of about this value. It is such a viscosity value (between 6 and 7) which I attribute to echinoderm ova.

Viscosity of nucleus

CHAMBERS (5) states that the resting nucleus of marine ova "exists in the sol state," and describes how it can be pinched into two droplets which run together on coming into contact. KITE (15) says of the viscosity of the nucleus of the starfish egg: "With the exception of the nucleolus, the nuclear substance is all in the sol state." Of the nucleus of *Amoeba proteus*, however, he says: "The whole of the nuclear substance is a highly rigid and granular gel, the minutest pieces of which show no appreciable change when dissected out in distilled water."

Although one must be very cautious in assuming that all protoplasm is possessed of the same physical properties as that particular protoplasm examined, yet on general principles it would seem that it is hardly likely that the nucleus of an ovum is very liquid (that is, a sol), and that of *Amoeba* a highly rigid gel. My observations on the nucleus of *Amoeba* show that its viscosity is also low, as is the viscosity of nuclei of marine ova (as stated by KITE and CHAMBERS). Results based on the examination of isolated pieces of protoplasm are very uncertain, and that minute pieces of the nuclear substance undergo no change when dissected out into water is quite untenable.

The nucleus of *Amoeba* is rather liquid, but by no means watery, for it possesses a slight degree of viscosity. It is apparently in the sol state. The nucleus when freed from the organism increases in consistency, sometimes slowly and sometimes rapidly. The extremely viscous and in all probability partially degenerate substance of an isolated nucleus is very coherent and elastic, capable of being stretched into fine, barely visible threads. Ultimately the isolated nucleus degenerates into a granular coagulum.

Changes in protoplasmic consistency

While protoplasm may be more or less permanently of a definite viscosity (in such forms as *Euplotes*), yet it does undergo reversible changes. For example, the ectosarc of *Amoeba* is characteristically of a high consistency, yet at the tip of an advancing pseudopodium it becomes temporarily very liquid. Also, in Myxomycetes and bread mold the quiescent protoplasm is very

dense, while the streaming plasma is much more dilute. The change from one state to the other is dependent upon (or at least coincident with) physiological (or physical) activity.

The changes in consistency so far considered have had to do only with the one phenomenon of streaming. There are several other factors which bring on changes in protoplasmic consistency, however, such as development (growth), reproduction, mitosis, and pathological conditions. These changes may be in one direction only, and relatively permanent, as the change from a liquid state to a highly viscous one in the process of fruiting in Myxomycetes; or they may be periodic and reversible. The latter type is exemplified in the changes which accompany streaming. Of the former type the gradual increase in consistency during development of the egg of *Fucus* is an example.

DEVELOPMENTAL CHANGES IN VISCOSITY.—The developmental change in viscosity has been fully described in my former publication (**23**). The protoplasm of young uninucleate oogonia is of liquid consistency (v.v. 3). I think this value is more accurate than the "very liquid" one given in the former publication referred to. Nearly mature oogonia, in which division into 8 eggs is just complete, are of slightly viscous consistency (v.v. 4). As the eggs near maturity they increase to the rather viscous stage (v.v. 5), and the fully mature, normally discharged egg is decidedly viscous (v.v. 6). It is interesting to note that this progressive increase in consistency is coincident with a decrease in physiological activity. The young oogonium with protoplasm of liquid consistency is in a state of active growth, while the decidedly viscous ripe egg is in a more or less quiescent state awaiting fertilization.

REPRODUCTIVE CHANGES IN VISCOSITY.—I have already referred to the increase in viscosity of a Myxomycete plasmodium as it prepares to fruit. The liquid density (v.v. 3) of the active vegetative stage becomes, when inactive, extremely viscous (v.v. 8), and, on preparing to fruit, increases to a gel consistency.

CHANGES IN VISCOSITY DURING MITOSIS.—Division following fertilization in marine ova brings on very decided changes in viscosity. Earlier work on the ova of *Fucus* gave evidence of a marked decrease in consistency of the egg protoplasm within half

an hour after fertilization. Subsequent work on marine animal eggs has shown that the change in viscosity due to fertilization is not so simple a phenomenon as one might think from what can be observed in dissecting the egg of *Fucus*. The protoplasm of the ripe unfertilized egg is of comparatively uniform viscosity. Division, following fertilization, brings on pronounced regional differences in consistency. In the egg these differences are wholly obscured by the dense color of the chloroplasts.

The changes in protoplasmic consistency which are a consequence of fertilization can readily be determined in the dividing echinoderm egg. The following data were obtained principally from dissection of the ova of *Tripneustes*, but were in great part substantiated by subsequent work on the ova of *Echinarachnius*. My observations on the viscosity of the echinoderm egg during mitosis are less detailed than those of CHAMBERS (**6**). In brief, CHAMBERS finds that the sphere (the central transparent area of the aster of the mitotic figure) and the astral rays consist of a clear liquid of very low viscosity, while the surrounding cytoplasm is in the gel state, and that there is a "periodic reversal of the sol to the gel state and vice versa" during mitosis. The following data support, in the main, these findings of CHAMBERS.

The mature unfertilized sea-urchin egg is very viscous (v.v. 7) and comparatively uniform in its viscosity. Following fertilization a change in consistency soon takes place. With the first appearance of the aster there is an increase in viscosity of the peripheral cell cytoplasm. By peripheral protoplasm I refer to a broad outer zone as distinct from an inner core, and not to an ectoplasmic layer, a membrane, or the like. This increase in consistency of the general peripheral protoplasm is from the very viscous to the exceedingly viscous state. With the first appearance of the amphiasters there is a pronounced decrease in viscosity of the central region of the cell, and this condition is maintained throughout the intermediate stages of divisions (from middle prophase to late anaphase). Close examination shows that the dilute protoplasm in the center of the mitotic figure makes up the hyaline area surrounding each pole (the "hyaloplasm-sphere" of WILSON **28**).

The rays of the amphiasters, like the two polar spheres, are apparently also of dilute protoplasm. This thin hyaline substance which makes up the astral rays and polar areas is of liquid consistency (v.v. 3). Although quite dilute it is not watery. In connection with the low viscosity of the hyaline rays it is interesting to recall the theories which have been advanced pertaining to the flow of the substance of which the rays are composed. AUERBACH was probably the first to advance the theory that the rays were currents of a protoplasmic substance. Others, notably FOL, have advocated a similar theory. WILSON (**28**) likewise upholds this theory with the statement that "no one . . . . can, I think, doubt that such a centripetal movement occurs, or that the clear hyaloplasm flows inwards to form the growing hyaloplasm-spheres." STRASBURGER (**24**) states that he, with FOL, looks upon the astral rays as "centripetal currents," to which STRASBURGER ascribes the function of "carrying to the astral body substance which serves as nourishment for the new nucleus."

The protoplasm peripherally located, and also that making up the wedge-shaped protrusions which alternate with the hyaline rays and thus give to the mitotic figure its starlike appearance, is all of high consistency, being very or extremely viscous (v.v. 7 or 8).

A very convincing demonstration of the fluidity of the substance of which the astral spheres and rays are composed was obtained through dissection and previous vital staining with neutral red. The eggs were placed in a very weak stain of neutral red at the time they were fertilized, and allowed to remain in the stain during division, which requires about an hour. The clear liquid substance of the spheres and rays stains a brilliant pink, while the surrounding highly viscous granular plasma takes on little if any stain. A stained ovum in the early anaphase of mitosis was torn until a small globule of protoplasm adjoining the egg had been formed. By pressure against the egg the protoplasm from it could be forced into the globule, thus enlarging the latter, and then by pressure against the globule the protoplasm could be forced back into the egg. The protoplasm which could thus again and again be made to flow from the egg into the globule, and vice versa, was the stained hyaline liquid substance of the astral

spheres, and its fluid condition as contrasted with the extremely viscous consistency of the peripheral protoplasm was very noticeable. It was also strikingly evident that the clear liquid substance did not mix with the granular protoplasm during several minutes of kneading of the egg contents.

The interesting question arises, What is this clear liquid substance which makes up the astral polar areas and rays? WILSON (**27**) refers to it as hyaloplasm (matrix).[11] CHAMBERS (**6**) is noncommital and refers to the contents of the sphere "as the sphere substance or sphere liquid." If the sphere and ray substance is hyaloplasm (matrix), it is very likely a modified, and perhaps greatly modified, form of it, and therefore strictly not hyaloplasm. The unusual circumstances under which it is produced rather suggest that it is at least a modified form of the matrix. This same question arose concerning the identity of the exuded globules of clear substance from Myxomycete plasmodia. Whether the sphere substance is a secretion, which does not seem likely, or an extravasation of one of the many complex phases of the living colloidal system cannot be determined.

With the coming of the telophase of mitosis and the disappearance of the aster, the viscosity of the central protoplasm of the egg rises from the low value of the sphere substance (v.v. 3) to a viscosity value of 6, and with the completion of division we have in each daughter cell of the embryo a general protoplasmic consistency identical with that of the egg before fertilization (v.v. between 7 and 8).

PATHOLOGICAL CHANGES IN VISCOSITY.—The changes in viscosity so far considered have all been of living and normal protoplasm. In determining degrees of viscosity of protoplasm it has been necessary to guard carefully against misinterpretations due to the readiness with which protoplasm alters its consistency as a result of dissection and aging, both of which bring on pathological changes which inevitably result in an increase in viscosity.

[11] "The substance thus flowing inwards I shall for the present designate simply as hyaloplasm (equivalent to the 'cyanoplasm' of MORGAN), and I believe it represents wholly or in part the interalveolar or continuous substance lying between the alveolar spheres"—(WILSON **27**).

Prolonged dissection always ultimately causes an increase in the consistency of protoplasm, unless rapid dissolution first takes place. The rate of increase in viscosity varies greatly in different types of organisms, and even in different individuals of the same type. It is surprising how much dissection protoplasm will often tolerate without showing any increase in viscosity or sign of degeneration, but no protoplasm will endure churning by micro-dissection needles indefinitely. The outcome of such ill treatment may be rapid disintegration or a pronounced increase in viscosity, probably gelation. HYMAN (13) states that "the injury of cutting may completely alter the physical state of protoplasm, probably in the direction of liquefaction." This is not true unless the injury is sufficient to cause complete degeneration, that is, death. Injury not resulting in death always causes an increase in viscosity of the protoplasm. In the advent of death liquefaction does first take place, followed by coagulation.

The injury to an organism undergoing dissection may be general or local; in either case an increase in viscosity results. If the increase in consistency is local, the injured region may be discarded, apparently by the organism, although actually the living organism plays only a passive part, or it may be reabsorbed, by reverting to the sol state. If the increase in viscosity is general and pronounced, death follows.

An *Amoeba* usually shows little change in viscosity over that of the normal quiescent stage as a result of quite some minutes of dissection. Finally, however, either a sudden and pronounced gelation takes place, or, more often, rapid disintegration results. In Myxomycetes the rate of change in consistency due to physical disturbance is slow and gradual. Ultimately, protoplasm subjected to much dissection will always coagulate, unless preceded by rapid dissolution. A coagulum thus formed at death can be cut up into pieces which exhibit none of the properties of the living substance, such as glutinosity, plasticity, elasticity, etc.

Marine ova are subject to the same pathological changes in viscosity as a result of dissection and aging. The increase in consistency from dissection takes place more rapidly than in Myxomycete protoplasm. Here again the degenerate protoplasm ultimately

coagulates (rapid or slow dissolution may follow as well as precede coagulation). The coagulum is coarsely granular in appearance, suggesting a granular precipitate.

Brownian movement of protoplasmic particles

It is rather surprising how little the presence of Brownian movement of particles in really living protoplasm is appreciated. It is usually said that protoplasm, because of its comparatively high viscosity, will not permit Brownian movement of its particles, and that only in vacuoles, whose contents is a dilute sap, is Brownian movement of particles to be observed in a living cell. The classical example of this is the terminal vacuoles of the desmid *Closterium*. It must be borne in mind that I have reference here only to microscopically visible particles, such as make up the granular plasm, and not to ultramicroscopic colloidal particles which are in a constant state of vibration in probably all liquid protoplasm. When workers refer to "the Brownian movement of particles" (2) contained in protoplasm, they do not always explain whether the reference is to microscopic or ultramicroscopic particles. Usually it is apparently the latter. Brownian movement of suspended microscopic particles in protoplasm cannot be seen in by any means all cells or organisms, but it is to be observed in the dilute endoplasm of some ciliates, in the liquid protoplasm of streaming Myxomycetes, and to a striking degree in one of the most studied of organisms, *Amoeba*.

In a quiescent *Amoeba* the number of particles exhibiting Brownian movement is small, and the amplitude of the movement is short. In an active *Amoeba*, the protoplasm of which is more dilute, all particles except the largest droplets exhibit Brownian movement, the motion varying inversely as the size of the particle (4 μ seems to be the maximum size of particles capable of the vibration, according to YOCOM 30), and the amplitude of vibration is relatively large. In the liquid endoplasm of the ciliate infusorian *Euplotes* the suspended particles are frequently in a state of vibration, especially when cyclosis is taking place.

The protoplasm of *Amoeba* in which Brownian movement is to be observed is of rather liquid consistency, and when this proto-

plasm increases in viscosity coincident with a decrease in activity, both the number of particles exhibiting Brownian movement and the amplitude of vibration are lessened. This fact is expressed in the physical law which states that the amplitude of vibration of a particle of a given size is inversely proportional to the viscosity of the dispersion medium (8). This law is further supported by the fact that in highly viscous living protoplasm no Brownian movement is to be seen.

Brownian movement of particles is so characteristic a phenomenon of degenerate protoplasm that I was led to look upon it as an "unfailing criterion of degeneration" (23). Such a conclusion, in view of the presence of Brownian movement in living protoplasm, is not justifiable. It is true, however, that one of the first signs of degeneration in protoplasm, which in the living normal condition shows no Brownian movement, is the instant assumption of a marked oscillating motion of the protoplasmic particles. The surprising thing, however, is that this Brownian movement is actually taking place in an apparently highly viscous mass. Careful dissection will reveal the fact that the degenerate protoplasm has gelated only at the surface, sometimes to an appreciable depth, while the interior of the mass is very dilute. It is in this watery degenerate protoplasm that the suspended particles are in vibration.[12] Death, therefore, has resulted in a liquefaction, probably due to excessive imbibition of the protoplasm. This fact is not generally realized, primarily because the watery condition is only temporary, since ultimately the whole of the protoplasm becomes a rigid coagulum. GAIDUKOV (9), for example, in describing the death changes which take place in the living emulsion, says that "with death of the plasma a coagulation results, which in the case of slow death, is a precipitation, and of sudden death (by fixation), a congealing." I wish again to emphasize the fact that this liquefaction of the protoplasm is a consequence of death, and not of mere injury. Injury, not resulting in death, invariably produces an increase in viscosity. It is not true, therefore, that Brownian movement of microscopic particles in protoplasm ends with death (2). On the contrary, it is

[12] Diffusion of the dilute protoplasm is prevented by rapid gelation at the surface.

frequently set up as one of the consequences of a death phenomenon, that is, temporary liquefaction. When GAIDUKOV states that at death "the motion of the particles ceases," he undoubtedly has reference to ultramicroscopic colloidal particles, and even here this cessation of motion must sometimes be the ultimate and not always the immediate result of death.

The consistency of concentrated laboratory glycerine (specific gravity 1.25) is just high enough to prevent a visible vibration of carmine particles suspended in it. It is not always evident that the viscosity of living protoplasm in which no Brownian movement is to be seen is as high as that of concentrated glycerine. As a general rule, however, it can be stated definitely that protoplasmic particles in a medium of high consistency are not in vibration, while those in very liquid protoplasm are. As a criterion of the viscosity of protoplasm as a whole I do not regard the occurrence or non-occurrence of Brownian movement as very accurate or conclusive.

Summary

1. Protoplasm is a polyphase emulsoid system.

2. Physical structure and not viscosity determines the sol or gel state of an emulsion. Consequently, while protoplasm undoubtedly exists sometimes as a sol and sometimes as a gel, yet sol and gel as descriptive terms of the physical state of protoplasm must be used with great caution when viscosity is the only criterion.

3. The viscosity of protoplasm ranges from a degree slightly more than that of water to the firmness of a fairly rigid gel.

4. While a certain degree of viscosity may characterize the protoplast as a whole, the latter is always more or less divided into regions, whether larger general protoplasmic regions such as ectoplasm and endoplasm, or smaller localized centers of protoplasmic activity such as nucleus and chromatophores, which differ in viscosity from the protoplasm as a whole.

5. Some protoplasmic regions do not noticeably vary in their consistency, but the viscosity of a protoplast as a whole generally varies considerably within a rather wide range.

6. Some of the factors influencing changes in protoplasmic consistency are periodic changes in physiological activity, development, reproduction, mitosis, injury, and death.

7. Streaming protoplasm is less viscous than quiescent protoplasm.

8. Young active protoplasm increases in viscosity as it matures and becomes less active.

9. As a Myxomycete plasmodium prepares to fruit, its consistency becomes very high.

10. During mitosis there are very marked regional changes in viscosity.

11. Physical disturbance usually causes a pronounced increase in viscosity, although the rate of increase varies greatly in different individuals.

12. At death protoplasm frequently becomes temporarily very dilute, probably the result of excessive imbibition. Ultimately the degenerate protoplasm coagulates into a solid granular mass, if rapid dissolution has not preceded coagulation.

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GROUPING AND MUTATION IN BOTRYCHIUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 273

CHARLES J. CHAMBERLAIN

(WITH ELEVEN FIGURES)

Ever since the appearance of CHRYSLER'S[1] paper, claiming that the fertile spike of *Botrychium* represents a pair of fused pinnae of the vegetative leaf, I have been interested to note the peculiarities in the spore-bearing portions, upon which he relies for a part of his evidence. Two fertile spikes in the position of the lower pair of leaflets, spikes in which the two component leaflets are incompletely fused, sporangia on the second pair of leaflets in addition to the sporangia of the fertile spike, and occasional sporangia on vegetative leaves were found during vacation field studies of *Botrychium obliquum* and its varieties. CHRYSLER'S claim needs no support, his evidence both from field study and anatomy being so convincing that, for years, we have treated these forms as a mere family, Ophioglossaceae, under the Filicales.

However, the field studies, carried on for several years during September vacations in Ohio, at Oberlin, Sullivan, Cleveland, and Birmingham, together with a few observations at Osborn, Indiana, and at Fort Sheridan, Illinois, impressed upon me that one scarcely ever finds isolated plants of *Botrychium*. They are almost invariably grouped; even when there seems to be an isolated plant, others can usually be found in the immediate vicinity.

In *Ophioglossum* there is abundant vegetative propagation by branching of the rhizome, so that not only are the plants grouped, but the plants of a group are more or less connected. In striking contrast, *Botrychium* shows no vegetative propagation. The rhizome scarcely ever branches and, when it does, the branch is not likely to become separated and form a new plant. Every plant comes from a prothallium. Consequently the distribution is

[1] CHRYSLER, M. A., The nature of the fertile spike in Ophioglossaceae. Ann. Botany **24**: 1–18. 1910.

entirely by spores; and since the prothallia are of the subterranean tuberous type, with an endophytic fungus, the prothallia develop only when the conditions for this rather unusual mode of development are present.

How far the spores might be carried is problematical. The grouping of plants indicates that most of the spores are not carried far, but when a plant is once established it becomes the center of a group.

At first I was interested only in the fact of grouping and in the size of the groups of *Botrychium obliquum* and *B. virginianum*. It was noted immediately that the groups of *B. virginianum* contained many more plants than those of *B. obliquum*, and that the groups were more closely associated. In counting plants and making plots, one soon learns to find specimens, especially the smaller ones, which easily escape notice, and the number of plants in a group is likely to be surprisingly larger than the average botanist would have guessed from a cursory examination.

The most closely associated groups, with the largest number of plants in a group, were found at the borders of rather open woods. Plants in the deeper woods, although likely to be large and vigorous, are not abundant.

During the Septembers of the past four years the grouping was observed, and a searching for prothallia developed some facility in recognizing young plants. In 1918 the plants of many groups were counted, especially at Sullivan, where *Botrychium* is exceptionally abundant; and in 1919 plots were made, showing not only the number and position of plants in a group, but also the relation of the groups to each other.

Botrychium virginianum is more abundant than *B. obliquum*, even when the two species are growing together under the same conditions. On the eastern border of a densely wooded tract at Sullivan, Ohio, where *B. virginianum* is more abundant than I have ever seen it in any other locality, prothallia were collected and observations were made for several years. The border of the woods is roughly marked by a rail fence, with but few trees on the eastern side and some trees removed on the western side, so that the woods end in what farmers call a "clearing." The plants and

prothallia are most abundant in the clearing, within 25 m. of the fence, becoming more and more scattered as the woods become denser, while at a distance of 200 m. west of the fence scarcely any plants are found. In this place plants are most abundant on little elevations caused by uprooted trees. When a large tree is blown down, the roots tear up a considerable quantity of soil, so that when the tree decays and disappears there remains a mound with a depression on one side of it. These little mounds of clay soil, scantily covered by moldy humus, seem to be exceptionally favorable places for the germination of spores and the growth of plants.

A few years ago, before any plots were made, the abundance of plants in this locality suggested counting the number on definite areas. These areas do not correspond exactly to the groups which were plotted later, because only the denser centers of the groups were considered, the more scattered plants at the borders being omitted. Some of the highest countings of plants on given areas are worth recording. On areas of 1 sq. m. there were 15, 20, 29, 30, 31, 42, 66, and 106 plants. In the last case the plants were very closely crowded, one cluster of 5 plants occupying a space only 3 cm. sq. On areas of 2 sq. m., there were counted 27, 43, 70, and 103 plants; on 4 sq. m., 112 plants; on 2 dm. sq., 7, 10, and 16 plants; and on 2.3 dm. sq., 8 and 21 plants.

In this clearing the groups were rather closely associated, being separated from each other by distances of 1–10 m., with only here and there a plant between. Many of these plants were small, some of them sporelings still attached to prothallia; but in any place where *Botrychium* is abundant there will be a goodly number of large plants. In such places white patches of the fungus can be seen by turning over the leaf mold.

Aside from noting the grouping and counting the number of plants in a group, little was done with *B. virginianum*. The same must be said of *B. simplex*, which was discovered accidentally at Osborn, Indiana, during a search for *Ophioglossum*. A group of a dozen specimens of this little *Botrychium* was found on an area of about 1 sq. m. So far as we know, this species has not been reported for the Chicago region.

The principal interest centered in *B. obliquum* and *B. dissectum*, which is often regarded as a variety of *B. obliquum*. There are other forms which taxonomists describe as varieties of *B. obliquum* and which may be as distinct and may have as definite a relation to the parent form as we believe *B. dissectum* has to *B. obliquum;* but we did not make any study of these forms, and in making plots and in counting we recognized only *B. dissectum*, and put all the rest—the varieties *oneidense*, *tenuifolium*, and *elongatum*—under the general name *B. obliquum*. Besides these varieties, which can often be identified with a manual, there are fluctuating variations, so that one who is not a professional taxonomist is tempted to call the whole assemblage *B. obliquum* and let the name cover *B. obliquum* and its derivatives.

B. obliquum does not occur in such large numbers as *B. virginianum*, the plants of a group being more scattered, with seldom more than a dozen plants on 1 sq. m. This difference in numbers and the difference in grouping is indicated in fig. 1. This plot represents an area of 33 by 40 m. The dots and the crosses of the diagram are all of one size, but the plants varied from sporelings still attached to prothallia up to large specimens. Where plants are so numerous, as indicated in the denser groups, not more than a quarter of them have fruiting spikes.

Why plants of *B. virginianum* should be so much more numerous than those of *B. obliquum*, when they are growing in the same situation, particularly when growing on the same spot, as shown in the lower right hand group in fig. 1, is not obvious. A million spores would be a very conservative estimate for the output of an average plant of either species, and in the largest plants the output probably reaches five or six million spores; but a comparison of the number of plants and the number of spores would indicate that far less than one spore in a million produces a plant which can be seen above ground. One might guess that spores which do not sift down immediately to a safe depth die very soon from exposure or only a little later from the winter's cold; but we have noticed that in the tropical rainy forests of southern Mexico, where *Botrychium* is abundant and where there would seem to be no danger from dryness or cold, prothallia are as difficult to find as in the United

States. It may be possible that differences in the sculpturing on the spore coats may facilitate or impede the penetration of the

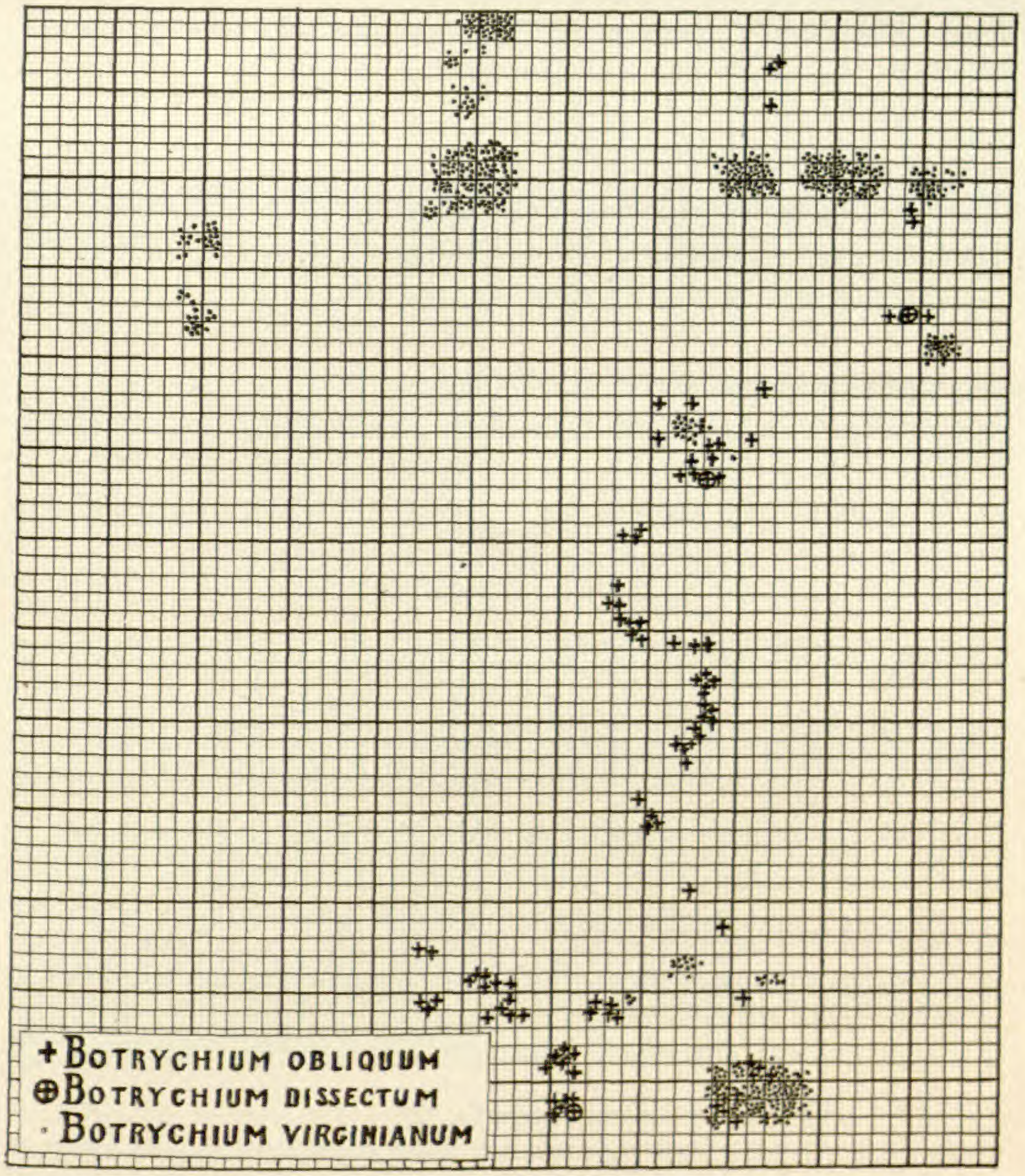


FIG. 1.—Plot 33×40 m. at Sullivan, Ohio, showing *Botrychium virginianum*, *B. obliquum*, and *B. dissectum;* each dot represents a plant of *B. virginianum;* each cross, a plant of *B. obliquum;* and each cross in a circle, a plant of *B. dissectum.*

spores to a favorable depth, or may favor or impede the absorption of water, and thus account for the larger number of plants of *B. virginianum.*

The principal study of *B. obliquum* was made at Oberlin, Ohio, in the cemetery, a part of which is sparsely covered by the original timber, while the rest is still more sparsely dotted with *Juniperus*, *Pinus*, *Thuja*, and *Cupressus*. Of the 24 groups which were counted

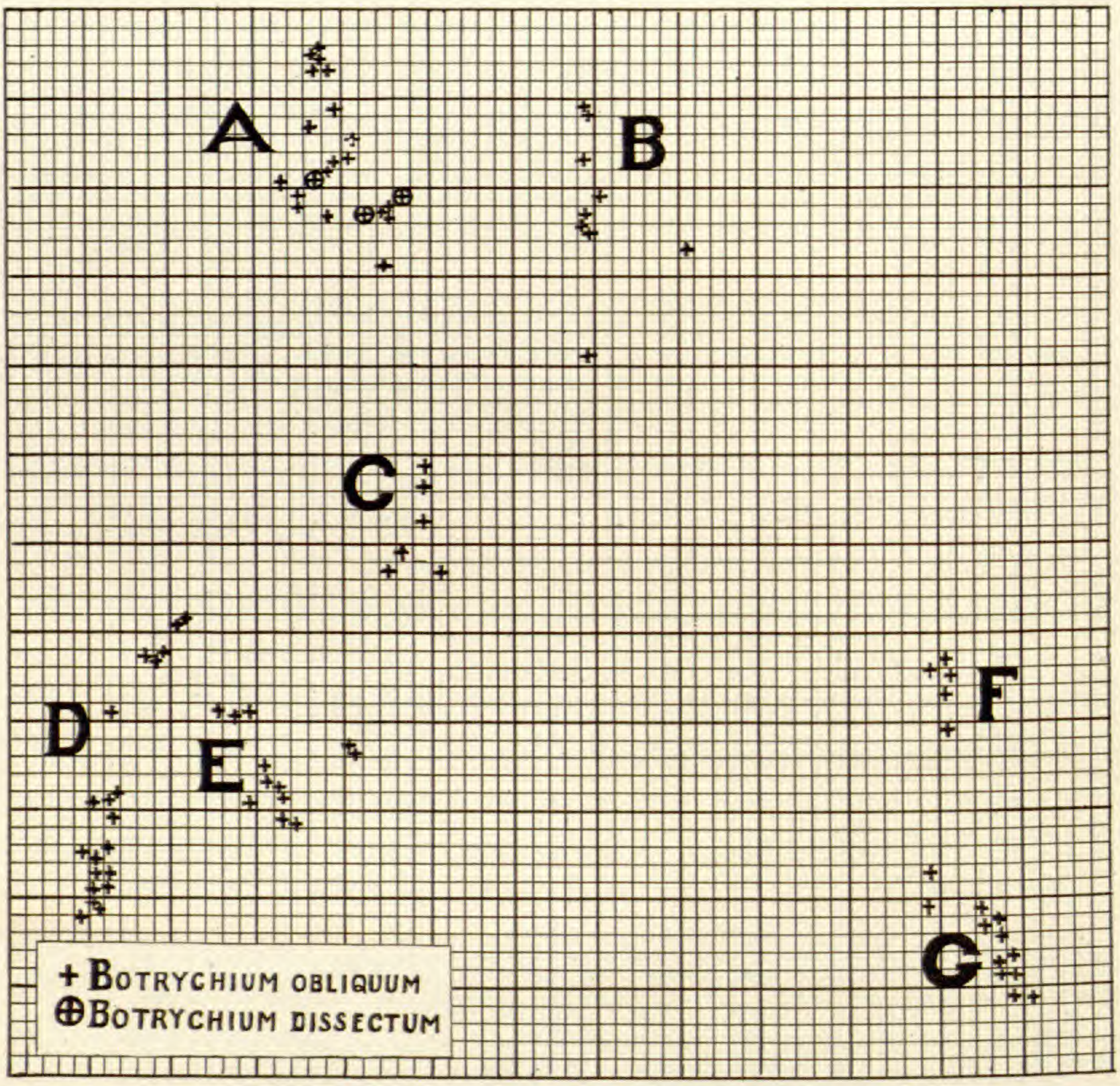


FIG. 2.—Plot about 40×43 m. at Oberlin, Ohio: distances between individual plants of a group approximately correct, but distances between groups *A* and *C*, *C* and *D*, *C* and *F*, and *F* and *G* about twice as great as indicated; there is no *B. virginianum* in this vicinity.

and plotted, 17 were at this place, 4 at Sullivan, 2 at Cleveland, and 1 at Pittsfield. A sample of the plotting at Oberlin is given in fig. 2.

It was from such detailed field studies as those shown in figs. 1 and 2 that we reached the conclusion that *B. dissectum* is a

mutant from *B. obliquum*. In ordinary cases such a conclusion would be tested by sowing the spores and growing the plants; but, so far as we are aware, no one has ever succeeded in raising prothallia of any species of *Botrychium* from the spore. Even if someone should find out how to grow prothallia and sporelings, it would

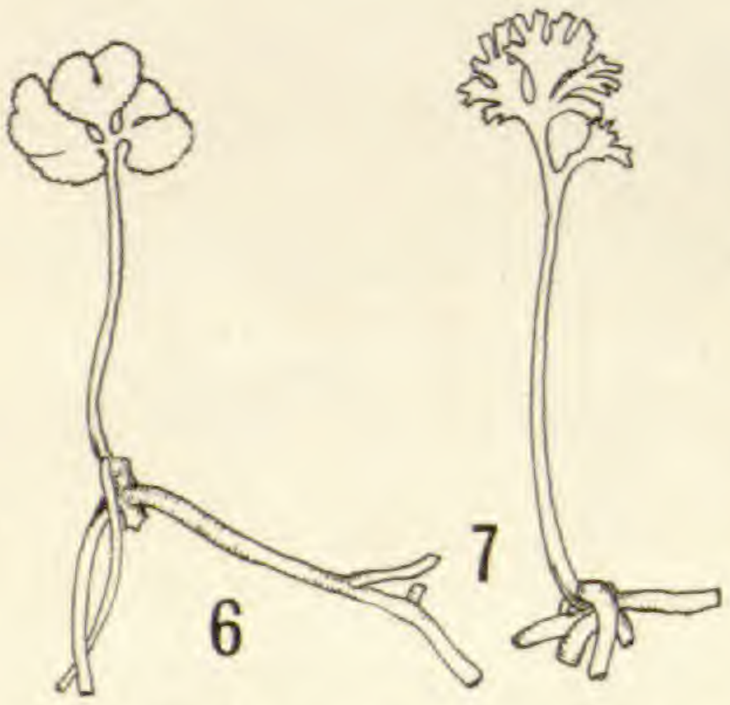
FIGS. 3–5.—Two vigorous plants of *B. obliquum*; plant of *B. dissectum* with typical leaf and roots, but with unusually good sporangia: small plant of *B. obliquum*.

take a long time to secure any results. How long the spore may rest before germinating is problematical; but even after it germinates it is probably a year or more before the prothallium reaches the fertilization stage. In adult plants the leaf is in its fourth year when it appears above ground. Consequently at least five years, and more probably six or eight years, would elapse between the

sowing of the spores and the appearance above ground of the first tiny leaf of the sporeling. We have seen one sporeling with a fertile spike bearing a few sporangia while still attached to the prothallium, but it is probable that ten or twelve years usually elapse between the germination of the spore and the development of a plant up to the spore-bearing stage. During the past twenty years we have made so many unsuccessful attempts to germinate *Botrychium* that we did not even try to test our theory by this method, but collected some circumstantial evidence which supports our conclusion that *B. dissectum* is a mutant from *B. obliquum*.

Before presenting the evidence it is worth while to call attention to the distinguishing characters of the two forms.

B. obliquum and its varieties have oblique leaflets with margins ranging from nearly entire to quite sharply serrate, sometimes doubly serrate, while *B. dissectum* has a leaflet, still oblique in topography, but so dissected that the specific name is very appropriate (figs. 3–5). This difference in leaves is recognizable even in the sporeling (figs. 6 and 7). The leaves of sporelings are simpler in outline than those of larger plants, but the general character of the margins is characteristic from the first, so that there is no danger of confusing the forms.



FIGS. 6, 7.—Sporeling of *B. obliquum;* natural size; sporeling of *B. dissectum;* natural size.

In *B. obliquum* and its varieties there is considerable variation in the shape of the leaflet and in the character of the margin; but, so far as the margin is concerned, the differences are confined to a greater or lesser degree of serration. The deepest serration of *B. obliquum* would not be mistaken for the deeply cut margins of *B. dissectum*. In *B. dissectum* there is also some variation in the margins, but the dissected character is always evident, the differences being in the extent of the dissection (fig. 8).

We are familiar, of course, with the great variations in the leaflets of cultivated ferns, where a single leaf may have leaflets with a nearly entire margin, leaflets deeply cut, and still others so

deeply cut that the bipinnate condition is reached. We do not regard *Botrychium* as a similar case, but believe that the differences in the margins of *B. obliquum* and *B. dissectum* are more like the differences in the margins of the leaflets of *Bowenia spectabilis* and *B. serrulata*, and like the differences in the leaf margins of *Dioon edule* and *D. spinulosum*. In these four cycads, the margins are so constant that they are reliable diagnostic characters.

The short subterranean stem, with the long-stalked leaf and spore-bearing portion with a still longer stalk, is similar in *B. obliquum* and *B. dissectum*.

The roots of *B. dissectum* are wrinkled and fleshy, like those of *B. obliquum*, and not at all like the slender roots figured in BRITTON and BROWN'S *Illustrated Flora*.

In general, *B. obliquum* is a larger plant than *B. dissectum*. At Oberlin the largest plant of *B. obliquum* measured 35 cm. in height, with a leaf 20 cm. in width, and the spore-bearing part of the fertile spike 15 cm. long. While this is not quite up to the limit in size recorded for the species, it is very large, and most individuals are much smaller. One plant of *B. dissectum* measured 28 cm. in height, but this is exceptional. The usual size of *B. dissectum* is about two-thirds that of *B. obliquum*.

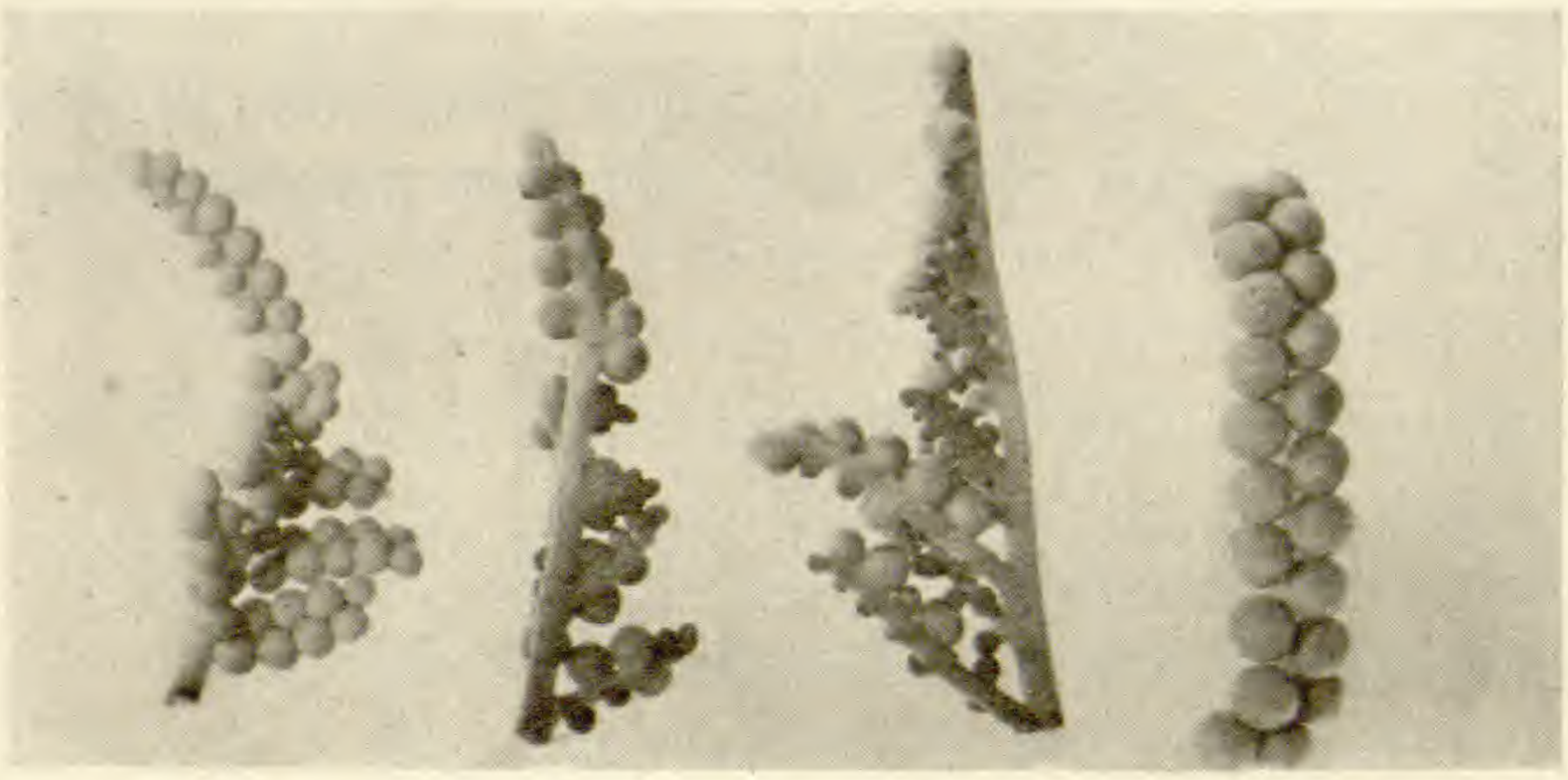
FIG. 8.—Leaflets from three plants of *B. dissectum;* natural size.

The most suggestive difference between *B. obliquum* and *B. dissectum* is seen in the fertile spike. The sporangia of *B. dissectum* sometimes look uniform and perfect, but somewhat smaller than those of *B. obliquum*. The difference in size, where the sporangia seem to be perfect, may be seen by comparing *A* and *D* of fig. 9.

However, most specimens show a considerable proportion of abortive sporangia which, even without sectioning, may be distinguished by their smaller size (fig. 9, *B* and *C*). The figure of *B. dissectum* in BRITTON and BROWN'S *Illustrated Flora* is evidently

drawn from a specimen with sporangia like those in fig. 9, *B* and *C*, and in this respect is characteristic.

Sections of sporangia, like those shown in fig. 9, *B* and *C*, show that the smaller sporangia contain no spores at all (fig. 10). In most of these cases the sporangium wall is from 4 to 6 cells thick, with the inner layer not differentiated into a definite tapetum, and the outer lacking the anticlinal thickenings so characteristic of sporangia which produce even imperfect spores (fig. 11). In extreme cases the sporangium is a mere mass of parenchyma cells; in others, a narrow streak of mucilage indicates that sporogenous tissue had begun to form; in still others, like the one shown in



A *B* *C* *D*

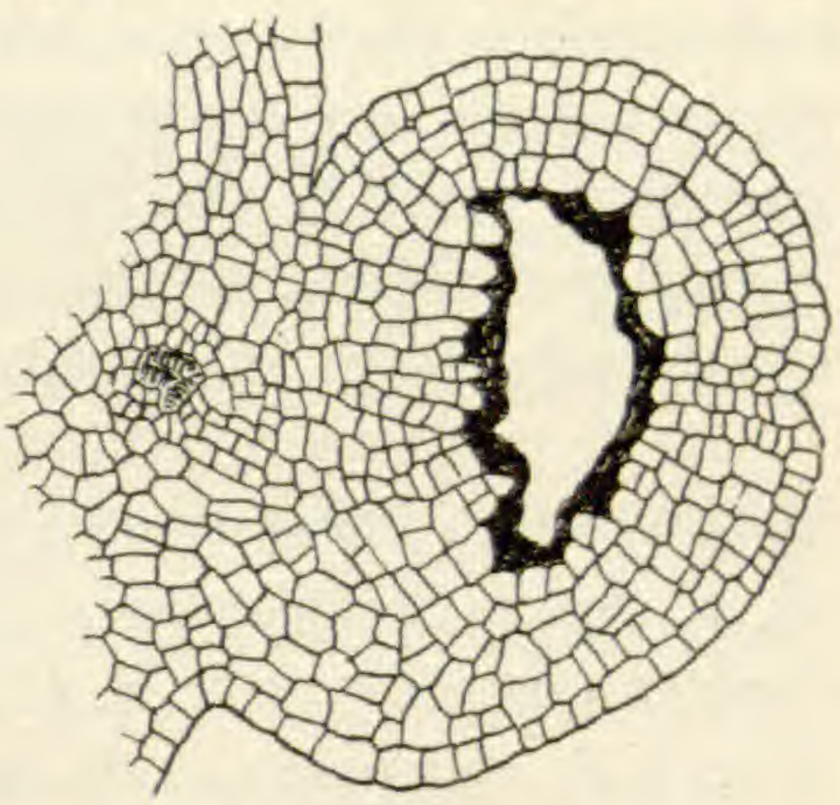
FIG. 9.—*A*, *B*, *C*, *B. dissectum*; *D*, *B. obliquum*; ×2

fig. 10, a considerable mass of sporogenous tissue has been formed; and in a few cases it could be seen that the mucilaginous mass consisted partly of imperfect, disorganizing spores.

In the apparently perfect sporangia of *B. dissectum* many of the spores are somewhat smaller than the average size for *B. obliquum*, and there are many spores which look as if they might be abortive. Fig. 11 shows six spores still floating in the tapetal plasmodium. The two spores at the upper left, one of them triangular in outline, are doubtless abortive; of the other four, only the one at the lower left has the full diameter of a normal spore of *B. obliquum*. The epidermal layer has anticlinal thickenings, as in normal sporangia, which dehisce and shed their spores.

It would be interesting to compare the reduction divisions of the two species, but the problem of getting material of *B. dissectum* makes such a comparison difficult, if not impossible. Even with good preparations of critical stages, the interpretation might be uncertain, for, judging by a few figures in *B. obliquum*, the *2x* number is well over 100, and the chromosomes are tangled and hard to count.

Such evidence as we have would indicate that *B. dissectum* is at least partially, and probably entirely, sterile. Unfortunately the natural test which would prove or disprove this theory—germinating the spores—cannot be applied until someone learns how to make these baffling spores germinate. If the spores of *B. dissectum* germinate, we do not see why this species should not occur in groups, like *B. virginianum*, *B. obliquum*, *B. simplex*, and probably the other species.



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11

FIGS. 10, 11.—Abortive sporangium of *B. dissectum*, with many-layered wall and mass of mucilage lining cavity from which sporogenous tissue has been resorbed; ×160: portion of sporangium of *B. dissectum*, showing spores of different sizes; triangular spore doubtless abortive; ×350.

In our opinion, the explanation of the occurrence and behavior of *B. dissectum* is that the species is a sterile mutant from *B. obliquum*. The principal facts supporting this theory are that *B. dissectum*, so far as I have observed in a five years' study, never occurs except in association with *B. obliquum*, and that there is no evidence that it reproduces itself.

It might be objected that mutants do not occur so frequently as *B. dissectum* would indicate, and it must be admitted that the total number of plants in our plots is not as large as one might wish in making ratios. The total number of plants in the twenty-four plots was 482 of *B. obliquum* and 19 of *B. dissectum*, a ratio

of 25:1. The ratio in the Oberlin group was 20:1; in the Sullivan group, about 48:1; and in the Cleveland group, 40:1.

However, in making any objections to the theory on the ground that *B. dissectum* occurs too frequently to be a mutant, it must be remembered that mutation in plants has been studied almost exclusively in angiosperms, which are heterosporous and which have comparatively low chromosome numbers. We are assuming that mutations occur in the mitotic mechanism, probably during the reduction divisions, so that the mutant, which one recognizes in the sporeling stage, is merely the result of a preceding phenomenon.

At first thought, someone might suggest that *B. dissectum* is a hybrid. What species could cross to give such characters as we find in *B. dissectum?* The mere question seems a sufficient answer, especially since *B. dissectum* is found when no other species except *B. obliquum* occurs in the vicinity. When we remember that the prothallia are of the tuberous, subterranean, saprophyte type, and not closely associated, the possibility of crossing seems very remote.

We believe the evidence is sufficient to raise a strong presumption that *B. dissectum* is a sterile mutant from *B. obliquum*.

UNIVERSITY OF CHICAGO

NEW OR NOTEWORTHY PORTO RICAN FUNGI

F. L. STEVENS

(WITH FOUR FIGURES)

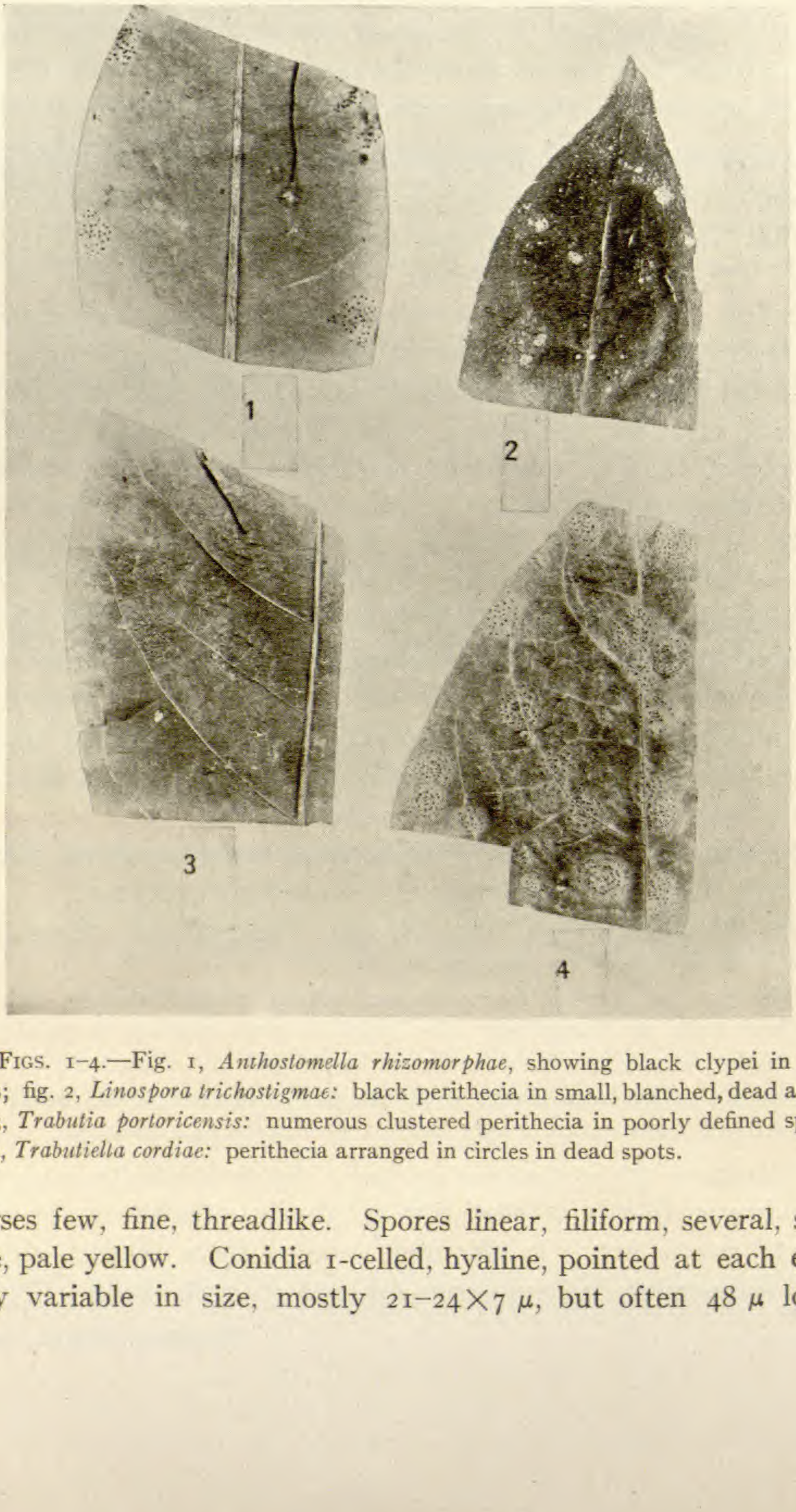
ANTHOSTOMELLA RHIZOMORPHAE (Ktz.) B. and V.—The spots caused by this fungus are pale to yellowish and are much swollen, so that they present a general aspect of insect galls. They are 0.5–1 cm. in diameter, circular, or when marginal more or less extended. Each spot contains several perithecia which are medially located in the leaf, that is, about equally distant from upper and lower epidermis. They are about 700 μ in diameter, thin-walled, the wall colorless, and have a coarse, hyaline mycelium extending throughout the spot, a black clypeus form in later stages occupying all of the space between the perithecium and the lower epidermis, which is 230–470 μ in diameter; the ostiole 40–50 μ in diameter. The paraphyses are numerous, threadlike, simple, septate, hyaline; asci oblong, stipitate, 150–175 or even 200 $\mu \times 50$ μ, thin-walled, 8-spored, inordinate. Spores oblong, 24–40 $\times$ 14–17 μ, brown when mature and 1-celled; the inner wall is uniform; the brown outer wall is pale yellow, wrinkled, and takes on very different appearances with different ages. Pycnidia are associated with the perithecia apparently on the same mycelium, oval, 125 μ across, 218 deep, with a thin clypeus; basidia short, simple; conidia oblong, pointed, obscurely 1-septate, pale straw colored, 3 $\times$ 10 μ.

On *Rhizophora Mangle*. Guanica, 2484; Penueles, 4559; Ponce, 8591, 9070; Cataño, 7607; San Jose Laguna, 9215.

The type is described on "coriaceous leaves" from Suriname, and may well have been on *Rhizophora*. The fungus is quite striking in appearance, but might be readily mistaken for an insect gall and thus overlooked by mycologists. The large spores with the several-layered coats, which give very striking appearances as they mature, are interesting structures.

Linospora trichostigmae, sp. nov.—Spots indefinite, 5–10 mm. in diameter, thickly studded with perithecia. Perithecia spherical, 150 to 200 to 250 μ in diameter, covered by a distinct clypeus and surrounded by a narrow (30–100 μ) pale zone. Clypeus

black, mostly epiphyllous, rarely hypophyllous. Ostiole irregular in shape. Asci cylindrical, 90–112×10–14 μ, thick-walled. Para-



FIGS. 1–4.—Fig. 1, *Anthostomella rhizomorphae*, showing black clypei in pale spots; fig. 2, *Linospora trichostigmae:* black perithecia in small, blanched, dead areas; fig. 3, *Trabutia portoricensis:* numerous clustered perithecia in poorly defined spots; fig. 4, *Trabutiella cordiae:* perithecia arranged in circles in dead spots.

physes few, fine, threadlike. Spores linear, filiform, several, septate, pale yellow. Conidia 1-celled, hyaline, pointed at each end, very variable in size, mostly 21–24×7 μ, but often 48 μ long,

and also often quite small, borne in cavities indistinguishable from the perithecia.

On *Trichostigma octandra*, Guayanilla, 5924.

The perithecia are conspicuous from above on account of the black clypeus, and from below because of the protuberance that they cause. At maturity the clypeus falls away, the contents of the perithecia drop out, and a hollow "poc-mark" cavity remains. The variability of the conidia is quite remarkable.

Trabutia portoricensis, sp. nov.—Spots approximately circular, densely set with perithecia, area of young spots not at all or but slightly discolored, tissue of old spots dead, tan colored. Perithecia conspicuous above, due to the shining black clypeus, from below by the protuberance which they cause. Perithecia opening epiphyllous, clypeus black, 80–95 μ in diameter. Ostiole central, 10–15 μ in diameter. Perithecium central in the mesophyll. Asci irregular, thin-walled, 8-spored, inordinate, 68×17 μ. Paraphyses many; spores filiform, oblong, obtuse, 24×7 μ, continuous, hyaline.

On *Cocolobis nivea*, Mayaguez, 3907a (type), 976.

Trabutiella, gen. nov.—Similar to *Trabutia*, but with the asci 16-spored. Similar to *Ditopella*, but distinguished from it by its clypeus. Type of genus the following.

Trabutiella cordiae, sp. nov.—Spots when young but slightly discolored; later the tissue dies and the spots are tan colored, or they may remain green longer than the adjacent healthy tissue. Spots definitely bordered, almost exactly circular, 5–10 mm. in diameter, with the perithecia in quite regular concentric rings. The black clypeus always epiphyllous, about 280 μ in diameter, or oblong and 240×500 μ. Ostiole 45–75 μ in diameter. Perithecia not visible from below, located in the mesophyll, 260–360 μ in diameter. Asci 85×17 μ, 16-spored, thin-walled, inordinate; spores oblong, pointed at each end, 20×3.5 μ, continuous, hyaline.

On *Cordia collococca*, Añasco, 276 (type); Mayaguez, 6295, 3907; Patillo Springs, 5730; Jayuda, 3977a; Hormigueros, 215.

Hyponectria phaseoli, sp. nov.—Spots circular, 5–10 mm. in diameter, amphigenous, few to numerous, often coalescing, pale yellowish, translucent, border indefinite. Perithecia abundant, immersed, translucent; when mature, with distinct protruding

is essentially a new book rather than a second edition of that formerly noted.[3]

Beginning with a sketch of the history of the botanical exploration of New Zealand, and noting the landmarks in her botanical literature, the author instructs the reader regarding the fundamental concepts of plant ecology in clear and simple terms, preparing him to follow appreciatively the description of New Zealand plants, not only considered as individuals, but as grouped in communities. Separate chapters are devoted to the vegetation of the sea coast, the inland waters, the mountains, and the outlying islands, as well as to the forests, the scrub, and the grasslands. The descriptions are so good that not only may they be understood by the New Zealand school boy (for it is an authorized textbook in the public schools), but they may also serve to furnish a graphic picture of a unique vegetation to the ecologists of other lands. For the latter the separation of New Zealand into botanical districts and the analysis of the flora into its different elements is particularly interesting. Moreover, the botanist is not at a loss to know what plants are intended by their common designations, for the scientific names always follow. In this, as well as in the use of many excellent illustrations, the volume may well be regarded as showing a standard of excellence seldom attained.—GEO. D. FULLER.

MINOR NOTICES

Cactaceae.—The second volume of the elaborate monograph of Cactaceae by BRITTON and ROSE[4] has just appeared. In fullness of description and wealth of illustration it leaves nothing to be desired. The colored plates are particularly noteworthy. The volume includes two of the eight subtribes of Cereae. In subtribe Cereaneae, 38 genera are recognized, including 16 new genera as follows: *Monvillea, Espostoa, Browningia, Stetsonia, Corryocactus, Erdisia, Leocereus, Dendrocereus, Machaerocereus, Brachycereus, Jasminocereus, Binghamia, Arrojadoa, Facheiroa, Zehnterella,* and *Neoraimondia.* There are also described 40 new species distributed among the various genera. The subtribe Hylocereanae includes nine genera, *Wilmattea, Mediocactus,* and *Deamia* being new, and 48 species, 6 of which are new. The monograph is an impressive illustration of the extensiveness of the cactus flora and its need of taxonomic reconstruction.—J.M.C.

Flora of Jamaica.—The fourth volume of FAWCETT and RENDLE'S *Flora of Jamaica*[5] continues the Dicotyledons, which began in the third volume,

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[3] BOT. GAZ. **52**: 159. 1911.

[4] BRITTON, N. L., and ROSE, J. N., The Cactaceae. Vol. II. Publ. Carnegie Inst. no. 248. pp. vii+239. *pls.* 40. *figs.* 305. 1920.

[5] FAWCETT, W., and RENDLE, A. B., Flora of Jamaica, containing descriptions of the flowering plants known from the island. Vol. IV. Dicotyledons (Leguminosae to Callitrichaceae). 8vo. xv+369. *figs.* 114. Published by the British Museum. 1920.

published in 1914.[6] The volume includes 13 families, much the largest ones being Leguminosae and Euphorbiaceae, with 50 and 30 native genera respectively, the remaining 11 families being represented by 34 genera. The contrast with north temperate floras is striking in the relative display of the various genera. For example, in the range of GRAY'S *Manual*, 11 native genera and 40 species of Euphorbiaceae are recorded, while in Jamaica this family is represented by 34 native genera and 111 species.—J.M.C.

Honey plants.—PELLETT[7] has listed alphabetically under their common names all plants known to contribute to the honey supply of the country. Simple descriptions and many rather good illustrations from photographs will enable the bee keeper to recognize the species in his particular locality, while scientific names insure accuracy. Some attention is also given to plants affording an abundant pollen supply. The volume should prove useful to the bee keeper, and interesting to the botanist or ecologist.—GEO. D. FULLER.

NOTES FOR STUDENTS

Taxonomic notes.—DRUMMOND and HUTCHINSON[8] have disintegrated the genus *Isopyrum* as ordinarily presented, separating from it 6 genera, *Asteropyrum* and *Paraquilegia* being described as new. The other separated genera are *Leptopyrum* Reichb., *Enemion* Raf., *Semiaquilegia* Makino, and *Souliea* Franch. There are 12 species retained in *Isopyrum,* one of which is new. This involves much shifting of nomenclature. For example, our common *Isopyrum biternatum* becomes *Enemion biternatum* Raf.

MOORE,[9] in continuation of his studies of the African flora, has described new genera in Erythroxylaceae (*Umbellulanthus*) and Icacinaceae (*Monocephalium*). In addition, 11 new species are described in these families and in Olacaceae.

WILDEMAN[10] has presented the African species of *Rinorea* (Violaceae), with full analytical keys and distribution, recognizing 106 species, 19 of which are described as new.

MOORE[11] has described the following new genera: *Homaliopsis* (Flacourtiaceae) and *Vaughania* (Leguminosae) from Madagascar, and *Hulemacanthus* (Acanthaceae) from Papua.

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[6] BOT. GAZ. **59**:334. 1915.

[7] PELLETT, F. C., American honey plants. 8vo. pp. 287. *figs. 152.* 1920. Hamilton, Ill. American Bee Journal.

[8] DRUMMOND, J. R., and HUTCHINSON, J., A revision of *Isopyrum* (Ranunculaceae) and its nearer allies. Kew Bull. 1920: no. 5. pp. 145–169.

[9] MOORE, SPENCER LE M., *Alabastra diversa.* XXXIII. 3. Miscellanea Africana. Jour. Botany **58**:219–226. 1920.

[10] WILDEMAN, E. DE, Notes sur le genre *Rinorea* Aubl. Bull. Jard. Bot. Bruxelles. **6**:131–194. 1920.

[11] MOORE, SPENCER LE M., *Alabastra diversa.* XXXIII. 1. Plantarum Mascarensium pugillus. 2. Acanthaceae Papuanae. Jour. Botany **58**:187–195. 1920.

ZIJP[12] has described a new genus (*Pseudodatura*) based on *Datura arborea* L. It had also been referred to *Brugmansia* by STEUDEL.

STAPF[13] has published a new genus (*Thellungia*) of Gramineae growing in Switzerland. It is related to *Sporobolus*, and the type is "an alien grown from wool refuse" about a mill. It is possibly of Australian origin, since numerous Australian grasses occur in the "rich alien flora" around the mill.

DUNN[14] has described a new genus (*Smithiella*) of Urticaceae from Eastern Himalaya. The genus is dedicated to Miss MATILDA SMITH, whose drawings and paintings for many years have been published in the *Botanical Magazine*, *Icones Plantarum*, and *Kew Bulletin*.

BROWN[15] has presented the results of his study of *Mesembryanthemum*, describing new species and also wishing "to demonstrate to future monographers the necessity for a thorough revision of the nomenclature of this interesting genus, as in the later monographs of it I have found that there are many errors in identification." Descriptions of 112 species are given, 62 of which are new. Two principal divisions of the genus are recognized, namely stemless species and those with stems, and under these divisions the species are distributed among 31 sections.

The Bolus Herbarium,[16] in continuation of its studies of African plants, has published 22 new species in various genera, an extensive list of flowering plants collected in Southwest Africa by the Percy Sladen Memorial Expedition (1915–1916), 6 new species of *Adenandra*, and 28 new species of *Agathosma*, both genera of Rutaceae.

OSTENFELD,[17] in connection with his studies of the West Australian flora, has published a revision of the following genera: *Triglochin* (7 spp.), *Crassula* (6 spp.), and *Frankenia* (15 spp.). New species are published in *Crassula* (1) and *Frankenia* (3).

VAN ALDERWERELT VAN ROSENBURGH,[18] in continuation of his studies of Malayan ferns, has discussed numerous species already credited to the flora, and described 50 new species. There are included also descriptions of 9 new species of *Selaginella* and a new *Lycopodium*.

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[12] ZIJP, C. VAN, *Pseudodatura*, nov. gen. Over. Natuur Tyds. Ned.-Indiè. 1920. 24–28.

[13] STAPF, O., *Thellungia*, a new genus of Gramineae. Kew Bull. 1920:96–99. *figs. 11*.

[14] Decades Kewenses. Kew Bull. 1920:210–212. *figs. 9*.

[15] BROWN, N. E., New and old species of *Mesembryanthemum*, with critical notes. Jour. Linn. Soc. **45**:53–140. *pls. 5–10*. 1920.

[16] Annals Bolus Herb. **3**:1–66. *pls. 2*. 1920.

[17] OSTENFELD, C. H., A revision of the West Australian species of *Triglochin*, *Crassula* (*Tillaea*), and *Frankenia*. Dansk Botanisk Arkiv **2**:30–55. *pl. 4. figs. 19*. 1918.

[18] VAN ALDERWERELT VAN ROSENBURGH, C. R. W. K., New or interesting Malayan ferns. Bull. Jard. Bot. Buitenzorg **2**:129–186. 1920.

DIXON[19] has published a report on the mosses of the Dümmer-Maclennan Expedition to Mount Elgon in 1918, and also on a small collection from the Aberdale Mountains. In the larger collection 46 species are reported, representing 32 genera. Ten new species, in as many genera, are reported for the smaller collection, and a new genus is established (*Kleioweisiopsis*) in Pottiaceae.

LANGE, [20] in his third part of the Agarics of Denmark, presents *Pluteus* (15 spp.), *Collybia* (28 spp.), and *Inocybe*. (47 spp.). Only 4 new species are described (in *Inocybe*), but there are many transfers, based upon new conceptions of species and genera.

SETCHELL and GARDNER[21] have described 16 new species of marine algae, distributed among 9 genera, one of which (*Internoretia*) is new. It is an endophyte, "growing within the membranes of *Porphyra Naiadum*." The same authors,[22] in the second part of their monograph of the marine algae of the Pacific Coast, have published the Chlorophyceae. The analytical keys, full descriptions, excellent illustrations, and complete bibliography, present the group in a most satisfactory way. The group is represented by 6 orders, 13 families, 34 genera, and 136 species. The largest genera are *Cladophora* (17 spp.), *Enteromorpha* (16 spp.), and *Ulva* (13 spp.).

BØRGESEN[23] has issued the third and fourth parts of his "Marine algae of the Danish West Indies," which continue the presentation of the Rhodophyceae. The two parts include 75 species, two of which are new, distributed among 29 genera. *Mesothamnion* is established as a new genus of the Ceramiaceae.—J.M.C.

Ovules and seeds of Thymeleaceae.—GUÉRIN[24] has investigated the anatomical structure of the ovule and seed of 27 genera of the Thymeleaceae. In the ovule the entrance to the micropyle is obstructed more or less by elongated cells which arise from the base of the stylar canal and become many-celled hairs. In some genera these cells are massed together and constitute a kind of obturator, which does not seem to hinder the penetration of the pollen

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[19] DIXON, H. N., Reports upon two collections of mosses from British East Africa. Smithson. Miscell. Coll. **72**: no. 3. pp. 20. *pls. 2.* 1920.

[20] LANGE, JAKOB E., Studies in the Agarics of Denmark. III. Dansk Botanisk Arkiv **2**:1–47. *pls. 1–3.* 1917.

[21] SETCHELL, W. A., and GARDNER, N. L., Phycological contributions. 1. Univ. Calif. Publ. Bot. **7**:279–324. *pls. 21–31.* 1920.

[22] ———, The marine algae of the Pacific Coast of North America. Part II. Chlorophyceae. Univ. Calif. Publ. Bot. **8**:139–374. *pls. 9–33.* 1920.

[23] BØRGESEN, F., The marine algae of the Danish West Indies. III and IV. Rhodophyceae (3 and 4). Dansk Botanisk Arkiv **3**:145–240, 241–304. *figs. 82* and *77.* 1917 and 1918.

[24] GUÉRIN, PAUL, Recherches sur la structure anatomique de l'ovule et de la graine des Thyméléacées. Ann. Jard. Bot. Buitenzorg II. **14**:1–35. 1915.

tube; in fact, in some genera these hairs seem to serve rather as a guide to the pollen tube. The fusion of the polar nuclei is tardy. The antipodals are always more than 3 in number, and in some genera very many more. The most striking feature of the ovule is the "hypostase" of VAN TIEGHEM, which is a clearly differentiated group of cells beneath the embryo sac, whose thin walls give the lignin reaction to stains. It occurs sometimes immediately beneath the embryo sac and sometimes deep in the chalaza. Its function is doubtful. The author raises the question whether it is not a distinct disadvantage in shutting off water conduction to the embryo sac.

In connection with seed development the author followed the changes in the tissues of the 2 integuments, finding that the inner integument contributes chiefly to the testa, but its persistent innermost layer separates from the testa and becomes a thin pellicle completely covering the embryo. In some of the genera in connection with seed formation, tracheae are developed in the periphery of the nucellus, connecting with the strands of the raphe and traversing the whole length of the nucellus. The author suggests that this is comparable to the tracheal nucellar mantle which characterizes the seeds of some of the Cycadofilicales. This feature has not been discovered before in the seeds of living plants.—J. M. C.

Tree growth.—MACDOUGAL[25] has issued a preliminary report describing briefly an instrument for recording the variations in diameter of tree trunks. Records extending over several months have now been made of individual trees of *Fraxinus arizonica*, *Pinus chihuahuana*, *P. radiata*, *Quercus agrifolia*, *Fagus grandifolia*, and *Platanus occidentalis*.

SHREVE[26] has added to these data a preliminary report of determination made on some stumps of *Pinus radiata*. The maximum increase in diameter for 10 years was 14 inches, while growth in height of 10 ft. for trees 12–15 inches in diameter has been known.—GEO. D. FULLER.

A new atmometer.—BATES[27] has devised a new atmometer which is said to have very nearly the same relation to wind and radiant energy as do the leaves of trees. A flat metallic chamber is constructed with a layer of moist linen between the upper and lower plates. The upper plate not only protects from rain, but also, possessing a blackened surface, absorbs radiant energy freely, while the lower plate is perforated to resemble the stomatal surface of leaves. Experiments have shown that the evaporation from this instrument follows the transpiration from small coniferous trees very closely.—GEO. D. FULLER.

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[25] MACDOUGAL, D. T., The dendrograph. Carn. Inst. Wash. Year Book for 1919. **18**:72–78. 1920.

[26] SHREVE, FORREST, Stem analysis and elongation in shoots of Monterey pine. Carn. Inst. Wash. Year Book for 1919. **18**:88–89. 1920.

[27] BATES, C. G., A new evaporimeter for use in forest studies. Mo. Weather Rev. **47**:283–294. *figs.* 3. 1919.

VOLUME LXX NUMBER 6

THE
BOTANICAL GAZETTE

DECEMBER 1920

FLOWER AND SEED OF HEDYOSMUM NUTANS[1]

J. GRAHAM EDWARDS

(WITH PLATES XXXIV–XXXVI)

The investigation of the seed development of *Hedyosmum* (Chloranthaceae) was undertaken at the suggestion of Professor DUNCAN S. JOHNSON. Of the 20 or more species of this genus, two species were studied. These two, *H. nutans* and *H. arborescens*, represent the largest of the three subgenera of the genus, so that the following results can perhaps be taken as characteristic of the genus as a whole. All of the figures used, except fig. 38, are from *H. nutans*. In the important features of the development of the embryo sac, perianth, etc., of *H. arborescens*, there is quite close agreement with those of *H. nutans*.

All of the material used was fixed by JOHNSON in chromo-acetic fixative.[2] The further work of imbedding, sectioning, and staining was done by the writer, except that figs. 41 and 44 were made from slides previously prepared by JOHNSON. The sections from which drawings were made were cut 7 μ thick. All sectioned material was stained with Flemming's triple stain and mounted in balsam.

The writer gratefully acknowledges the aid received from Professor JOHNSON, who inspected the slides from which drawings were made, read the manuscript, and gave helpful suggestions.

[1] Botanical Contribution from the Johns Hopkins University, no. 65.

[2] The material used was collected and fixed by D. S. JOHNSON in Jamaica in 1906, with the aid of a grant from the Bache fund.

Staminate flowers

The staminate flowers arise in short conelike catkins, consisting of 100 or more flowers each, one male catkin arising beside the female inflorescence near the end of the flowering shoot. Each male flower consists solely of a single, elongated, inverted, pyramidal stamen with a microsporangium at each of its four corners.

The microsporangia possess the usual epidermis, endothecium, tapetum, and several-layered archesporium (figs. 1, 2). From the spore mother cells, tetrads of microspores are produced in the usual way (figs. 3–5). The outer wall of the microspores (exine) is roughened by closely packed peglike tubercles 1 μ long and 0.5 μ in diameter (fig. 5). There are also present over the surface of the microspore 6 unthickened meridional bands, through one of which the pollen tube emerges (fig. 6, polar view). The inner wall of the microspore (intine) consists of a darkly staining layer of cellulose (fig. 5). The mature pollen grain has the usual vegetative and generative nuclei (fig. 5).

CLARKE (5) describes the stamens of the genera *Chloranthus*, *Ascarina*, and *Hedyosmum* as being two-celled, and before opening four-celled, due to a spurious, not always complete, dissepiment in the line of dehiscence. He says:

> The question referring to the structure of the anthers appears to have arisen entirely from those of *Chloranthus* itself as those of the other genera [of the family Chloranthaceae] are all of the ordinary two-celled character, or are spuriously four-celled from induplication at the line of dehiscence, a common occurrence with two-celled anthers; and in fact the four-celled structure is more apparent on a cross-section being made, both in *Hedyosmum* and *Ascarina*, especially the latter.

Neither SOLMS (12), BAILLON (1), EICHLER (6), BENTHAM and HOOKER (2), VAN TIEGHEM (13), nor ENGLER (7) refer to such peculiarity of structure as that mentioned by CLARKE. They describe the anthers as being of the ordinary four-sporangiate type. The writer finds his results agreeing with the later descriptions. In the sections of young anthers there are four distinct sporangia present from the outset of development (fig. 1).

Carpellate flowers

The carpellate flowers occur in compact cymelike inflorescences, several cymes in the axil of each leaf of the flowering branch.

Each flower is subtended by a hood-shaped bract, in the axil of which it arises. This bract, in early development, completely incloses the carpel and perianth (figs. 8, 12, 24).

When first observed, the young female flower consists of a short oblique column of tissue which is triangular in cross-section. On the side directly opposite the midrib of the bract the flower is distinctly lower than the sides next to the bract (figs. 8, 10).

PERIANTH.—The first step in the differentiation of the flower is the appearance of a ringlike upgrowth of the marginal portions of each of the faces of the triangular flower rudiment. This upgrowth is the young perianth. In the earliest stages seen this ring is clearly higher on the two faces lying to the right and left of the midrib of the bract. At first the outline of the floral rudiment as seen in cross-section is convex all around (fig. 11). Somewhat later, however, the outer tissue of the rudiment, that is, the tissue of the perianth, becomes much thicker on the three angles of the young flower, and a distinct depression is evident midway of the length of the flower on each side between the two angles (figs. 9, 10 *Po*). The depressed area bounded by the perianth is the wall of the carpel (fig. 17). This growth of the perianth continues from all directions, until in the ripe seed the wall of the carpel is overgrown by the perianth except for a small pore in each of the three flat sides (cf. figs. 9, 10, 25).

The character of the growth of the perianth is very evident from cross-sections (figs. 11 [upper and lower flower], 18) and from longitudinal sections (figs. 12, 14, 22). Only along a narrow strip, up and down each of the three corners of the ovary (figs. 11 [*X*, upper flower], 12 *Y*), and at a likewise narrow circular band around the upper third of the ovary (fig. 22 *Y*), are the tissues of the carpel and perianth continuous with each other.

A delicate vascular bundle is differentiated in the perianth at each of its three corners (figs. 11, 16). This vascular bundle divides into two near the top of the perianth, and these branches turn one to the right and one to the left, and later are found to be joined to an offshoot of a bundle of the carpel that pushes out into the perianth through the zone of attachment near the top of the ovary (fig. 22). The point of emergence of this carpellary bundle

is directly above the pore in the perianth on the flat side of the flower.

The perianth is never conspicuous either for its size or color. It is greenish, slightly yellow, or brown. It contains on its corners 15–20 layers of cells. The cells remain parenchymatous at first, but in the ripe seed they have rather thick walls and dense contents. The cells bordering on the pore become increasingly cutinized as development progresses.

Earlier observations on this ovary are the following: CLARKE (5) describes the female flowers as consisting of three sepals forming a tube not for the most part adhering to the ovary except at its base and apex where it becomes trifid. He says:

> There is a peculiarity occurring in the calyx of *Hedyosmum hirsutum* or an allied species (one of those in which the flowers are inclosed within thickened bracts so compactly that the apex of the calyx and stigma are alone discernible) which is probably quite singular: on removing bracteae, it is found that the calyx does not completely cover the ovary, but has three large loopholes, as it were, so that three flattened sides of the ovary are seen through it, although it is quite continuous at the angles and crowns it with its three segments as in other species.

SOLMS speaks of a triangular ovary and of the perigon as trifid at the apex, with the stigma sometimes alternate with the lobes of the perigon. BAILLON makes the following observation concerning the perianth: "Moreover, the apex of the ovary bears three short, thick, rounded wings alternating with its angles, two anterior and one posterior. Their morphological value is still uncertain." EICHLER speaks of a three-lobed half, or quite superior perigon. BENTHAM and HOOKER describe the tube of the perianth as grown to the ovary. VAN TIEGHEM says: "La fleur femelle, également nue, se compose d'un seule carpelle à style court renfermant un ovule orthotrope pendant; autour de la base du style, la paroi de l'ovaire se renfle quelquefois en trois bosses épaisses (Hed.)." ENGLER describes briefly the female flower as almost tubular and trifid at the corners. CLARKE has given a fuller description of the perianth, and each observer has mentioned some of the facts.

The question now remains as to the morphological nature of the perianth. The occurrence of three vascular bundles in the

perianth at the corners of the ovary, alternating with the three vascular bundles of the ovary itself, suggests that the perianth may be composed of three modified stamens standing one at each angle of the ovary. There is no definite evidence in favor of this view, since the earliest phases of the developing perianth show no essential peculiarities common to the perianth and to the stamens. The difficulty in accepting this homology is found in the unusual development of the upper portion of the perianth, the tissue of which in the earliest observed stages was in direct continuity with that of the ovary. In favor of such a view as just mentioned, perhaps, is ENGLER'S statement that the stamens of *Chloranthus inconspicuus* Sw. are united with a somewhat similar structure extending upward beyond the ovary about one-half its circumference.

Here also must be mentioned the fact that EICHLER (**6**, p. 7, in fig. 3 *B* and *C*) shows in *Chloranthus inconspicuus* an additional structure outside the three stamens, which he designates as a perianth. He says: "Das unterständige Ovar trägt an dem der Braktee zugekehrten Rande ein kleines, mehr weniger herablaufendes Schüppchen, das gewöhnlich als Andeutung eines Perigons betrachtet wird, und innerhalb dieses ein der Axe zugekrümmtes grosses dreilappiges Gebilde, das Androecium." Which of the two structures mentioned compares most closely in origin with the perianth of *Hedyosmum* must be determined by a study of the development of these structures in *Chloranthus*.

It seems clear from the preceding that the origin and growth of the structure termed perianth in *Hedyosmum*, especially in the presence of the lateral pore, differs from that of the perianth of any other angiosperm so far as is known to the writer.

OVARY.—The initial stage in the development of the ovary is that of a convex mass of cells (fig. 7). The outer margin of this dome of cells soon begins to grow more actively, thus leaving a depression in the center of the originally convex mass of cells (fig. 8, flower on left). The rapidly upgrowing ring of cells forms the wall of the ovary, while the central depression which is thus left at length becomes the ovarian cavity (figs. 8, 13, 18). By the continued upgrowth of the carpellary tissue about this depression, the ovarian cavity becomes deepened (figs. 13, 14 *OvC*).

Meanwhile the single nucellus of the ovary grows downward from one side (fig. 14), thus making the ovarian cavity appear crescentic in its upper half when seen in cross-section (fig. 18). This cavity in the lower portion about the hanging micropylar end of the ovule is completely circular in form (fig. 11 *OvC*, upper flower).

With the further growth of the ovary the ovarian cavity is extended upward by the stylar canal which opens out on the side of the style opposite the subtending bract (figs. 14 *SyC*, 22). The lower part of this ovarian cavity at the time the embryo sac is mature extends downward as an inverted conical chamber for half the distance to the base of the ovary (fig. 22). During the maturing of the fruit the cavity is filled by the single seed (fig. 42).

The style and stigma are developed by the continued growth of the upper margin of the carpellary ring, after the latter has closed together above the ovule to form the distinct stylar canal (figs. 14, 22). The stigma is formed solely from the longer lip of the upgrowing carpel (fig. 22). It consists, at the time of pollination, of a flattened triangular surface, the cells of which are parenchymatous. Later, in the ripe fruit, the mature stigma shrivels and disappears. The style is rather short. Internally it consists of two or more layers of elongated cells of small diameter which border on the stylar canal and probably serve as conducting tissue for the pollen tube (fig. 22). Around these layers of conducting tissue are 15 layers of other cells.

Early in its development the wall of the ovary consists of about 10 layers of cells (fig. 14). At the time the embryo sac is ripe the ovarian wall opposite the pore of the perianth is often 15 cells in thickness. Of the 15 layers of cells constituting the ovarian wall, only the 7 outer ones are appreciably specialized in structure. The epidermis becomes gradually cutinized as the embryo sac matures, and papillose cells arise in the exposed area where the perianth does not at first cover the carpellary wall. The cutin layer on the walls of these cells is thickest opposite the pore in the perianth, and becomes gradually thinner in the epidermal cells farther away from it.

At this stage, also, the 6 layers of cells within the epidermis of the ovary are strikingly irregular in contour. The 3 layers immedi-

ately adjoining the epidermis possess irregular cell contours, but their walls are not thickened. They are narrow and elongated. In the nearly mature seed they are not distinguishable from the epidermis, which is non-staining and presents a gelatinous appearance. The next 3 layers of cells adjoining these are thin in radial direction, while tangentially and longitudinally they are of considerably greater dimensions. By far the most salient peculiarity of these cells is the uneven thickening of the walls, which, when first appreciably thickened, are 2–3 μ thick. In the mature seed they are 7–8 μ thick. In the latter case the entire cell cavity is occluded and there is no trace of a nucleus, whereas in the younger ovary, with slightly thickened walls, the nuclei are distinct and remain so until the 10-celled stage of the embryo. The uneven thickening is shown in places by relatively large pits or thin areas (fig. 23 *Pi*). The substance constituting the thickened portions of this wall was found to be cellulose or a cellulose-like substance. These 3 layers of thickened cells doubtless serve to protect the seed. The remaining tissue of the ovarian wall, aside from the vascular bundles, consists of thin-walled parenchymatous cells.

Just within the layers of thickened cells the primary vascular bundles of the carpel are found. There are three of these, one growing up from the base of the ovary along the middle of each flat face (figs. 11 *VB*, 21 *VB-ov*, 42). These bundles are laid down very early in carpellary development and extend for a considerable distance into the tissue of the bract. These bundles extend upward in the wall of the ovary to the level of the chalaza of the ovule, where each bundle divides into two. One of each of these turns inward and bends downward to enter the base of the ovule (figs. 21, 22). From one or perhaps sometimes from each of the three nucellar branches there arises a strand which passes upward to form the single vascular bundle of the style. The second branch of each of the primary carpellary bundles turns outward, away from the nucellus, and passes out from the tissue of the carpel into the perianth through the zone of attachment near the top of the ovule, where the tissue of the carpel and perianth are continuous (figs. 14, 21, 22). Immediately after entering the perianth this perianth branch of the carpellary bundle turns upward to

end in the terminal lobe of the perianth. At either side as it turns upward this branch is joined by a branch from the perianth bundle itself (fig. 21 *x*). The presence in the wall of the ovary of 3 distinct bundles lends strong support to the view that the ovary is made up of 3 carpels, although no evidence of separate lobes could be distinguished at the upgrowing margin of the young ovary.

OVULE.—The nucellus of the ovule is initiated by the inward and downward growth, on the side opposite the bract, of the subepidermal cells of the wall of the ovary which border on the ovarian cavity (figs. 14, 18). The integuments arise soon after the carpel has closed in above the ovule to form the style and stigma. The inner integument starts as a ringlike outgrowth from the sides of the ovule near its middle. This is due chiefly to the activity of its epidermal cells (fig. 26). Soon after the inner integument appears, a second outgrowth slightly anterior to its base leads to the development of the outer integument (figs. 26 *OIn*, 28). These ringlike outgrowths continue growing downward around the ovule, that is, toward the micropyle, as it elongates. By the time the tapetal cell has divided, producing the 4 or 5 layers of cells constituting the tapetum, and the megaspore mother cell has come to occupy a central position within the nucellus, the inner integument has completely closed together above the nucellus to form the micropyle (fig. 28). The inner integument is longer than the outer from the outset of its development. At the time the embryo sac is ripe, the integuments extend considerably beyond the nucellus into the ovarian cavity, and each is 3 cells in thickness. From this time on neither the integuments nor nucellus change appreciably in appearance until the developing endosperm crushes them against the wall of the ovary where the integuments form the seed coat (fig. 42).

The 3 vascular bundles of the ovary can be distinguished entering the base of the nucellus almost immediately after its initiation (fig. 11, lower flower). The vascular bundle (indicated by dotted line in fig. 14) consists of cells which are elongating actively toward the nucellus, but the walls of which have not yet begun to thicken. When the embryo sac is mature the nucellus

consists of 7 or 8 layers of cells between the walls of the embryo sac and the outer surface of the nucellus.

The nucellus in the mature seed has been completely absorbed by the swelling endosperm, and there is left only the crushed remains of its cells against the two integuments, the inner of which is considerably thickened in the micropylar region (fig. 42).

Embryo sac

ARCHESPORIUM AND TAPETUM.—Of the group of hypodermal cells seen in the young ovule at the beginning of its development, two axial cells become distinguishable, owing to their larger size and larger nuclei, at the time the outer integument is clearly evident (fig. 26). These two cells are the parietal or tapetal cell above and the definitive archesporial or megaspore mother cell toward the chalaza (fig. 26). The tapetal cell divides early to form the 4 or 5 layers of parietal tissue found in the young seed above the sac (figs. 27–29). The cells of the tapetum thus formed do not change appreciably until crushed by the growth of the endosperm after the formation of the embryo.

The definitive archesporial cell is now considerably elongated and contains a large nucleus (fig. 28). This divides in the usual way to form 3 or more (frequently 4) potential megaspores, as is evident from the condition seen in figs. 29 and 30. The chalazal megaspore of the group is the one which becomes the functional megaspore and develops into the embryo sac, while the 2 or 3 micropylar ones degenerate and are later absorbed (figs. 29–31). The functional megaspore increases steadily in size during the degeneration of the non-functional megaspores, and then evidently begins to divide in the usual way, giving rise to 2, 4, and 8-nucleate stages. The first division of the mother cell nucleus was not observed, but the position within the embryo sac of the nuclei of its second division indicates a definite polarity after the first division, which is evidently maintained on through the subsequent divisions (figs. 31, 32).

The mature embryo sac is of the 7-nucleate type common to most angiosperms (fig. 33). The embryo sac continues to increase greatly in size, and the egg apparatus and endosperm nucleus are

seen to occupy typical positions within it (fig. 34). The endosperm nucleus at the time of its formation by the fusion of its polar nuclei and for some time thereafter contains two nucleoli. Later, however, but a single large nucleolus is visible (figs. 34, 35).

FERTILIZATION.—The writer has not investigated the details of the germination of the pollen grain upon the stigma, nor of the penetration of the pollen tube through the style into the ovarian cavity. From what was observed, it appears that the pollen tube enters the embryo sac in the usual way, displacing and disorganizing the synergids (figs. 35, 37).

The relative time of fusion of male nucleus with the egg was not determined with certainty, but it probably remains for some time in close proximity to the egg without fusion, during which time the endosperm nucleus proceeds to divide actively. The first division of the endosperm nucleus is followed immediately by the formation of a cell wall (fig. 36). Hence it may be said that the endosperm is "cellular from the outset of its development" (JOHNSON 9). The egg in *Hedyosmum nutans*, even when the endosperm has reached the 4-celled stage, is still undivided and uninucleolate (fig. 37). In *Hedyosmum arborescens* the fertilized egg is still undivided and binucleolate when the endosperm has reached the 10–12-celled stage (fig. 38). In the earliest stages of the 2-celled embryo seen, the number of endosperm cells is approximately 100. The endosperm continues to divide by longitudinal and transverse walls and rapidly encroaches on the substance of the nucellus (fig. 41). In the mature seed the endosperm has completely crushed and absorbed all but one layer of the nucellar tissue, so that the endosperm lies practically in contact with the inner integument (fig. 43). The antipodals persist unchanged for some time, but as the division of the endosperm continues they begin to degenerate, and finally disappear altogether. The embryo develops into a slightly elongated or oval mass of cells with a poorly developed suspensor (fig. 43).

The chief protection of the seed, except near the micropylar end, where the inner integument is considerably thickened, is afforded by the peculiar thick-walled, pitted cells of the carpel

(figs. 22, 23, 42). The ripe seed is somewhat oval in side view, but sharply triangular in cross-section.

Germination of seed

Observations of the germination of the seed were made in this laboratory by JOHNSON. It is of the type commonly occurring in endosperm-containing angiosperms. The small globular embryo differentiates gradually, and as the hypocotyl grows out of the nucellar region the cotyledons are extended on into the endosperm toward the chalaza. Here they remain until the stored food material is practically exhausted (fig. 44). Later they become freed from the endosperm, expand, and assume active photosynthetic functions.

Discussion

Certain peculiarities in the structure and development of the reproductive organs of *Hedyosmum nutans* which have been described suggest certain conclusions which may be drawn as to its phylogenetic origin. Correlated with these is the question of the comparative primitiveness of this plant in relation to other members of its family and order, and to other angiosperms.

CAMPBELL (3) has studied *Peperomia pellucida*, and JOHNSON has made detailed studies of this and 4 other species of the Piperaceae, as well as of representative genera of the other 3 families of the Piperales. Both investigators have called attention to certain facts bearing also on the relationship of the Chloranthaceae. It is on the basis of the views of these earlier investigators that the writer attempts to interpret the peculiarities occurring in *Hedyosmum*.

An interesting feature of the reproductive structures of this plant is the difference in character of the staminate and pistillate inflorescences. Whereas the staminate flowers occur in long-stalked ovoid catkins (see ENGLER and PRANTL **7,** fig. 13), the pistillate flowers, on the contrary, occur in sparsely flowered panicles. The occurrence of these two distinct types of inflorescence, along with the differentiation of the flowers into strictly staminate and pistillate ones, is to be noted in contrasting the

plants of this family with the other 3 families of the Piperales. In all three of the latter, the Saururaceae, Piperaceae, and Lacistemaceae, the flowers are hermaphrodite (except in *Piper Betel* L. var. *monoicum* C.DC. according to JOHNSON **10,** p. 716) and are arranged in distinct catkins. The occurrence of unisexual flowers is generally regarded as a mark of specialization within the family or order, and their presence in *Hedyosmum* indicates that this is one of the more specialized Piperales, rather than a very primitive one.

Again, the perianth of *Hedyosmum* is not of a primitive type. In fact it is a very unusual one. In so far as the writer has reviewed the literature on angiosperms in general, and the Piperales in particular, he has not discovered any type of floral envelope which is at all closely similar to it in its origin or mature structure.

CLARKE, who was the first to describe this perianth and to note its pores, does not discuss its origin, but refers to it as a calyx. SOLMS mentions a structure which he denotes as a perigon trifid at the apex. BAILLON says the apex of the ovary bears 3 short, thick, rounded wings whose morphological value is still uncertain. EICHLER speaks of a 3-lobed half or quite superior perigon. BENTHAM and HOOKER mention a tube of the perianth as grown to the ovary.

The endosperm, which is cellular from the outset of its development, finds its parallel in this respect in *Heckeria* and *Peperomia*, but differs from that of *Piper* (JOHNSON **8, 10**), the only other genus of Piperaceae whose development is known. Elsewhere among dicotyledons the type of endosperm formation found in *Hedyosmum* occurs only in highly specialized forms (JOHNSON **9**). The families in which this is found are placed by ENGLER in the higher orders of the Archichlamydeae and Metachlamydeae. It would seem, therefore, that the conclusion reached by HOFMEISTER, STRASBURGER, and HEGELMAIER, that the structure and development of the gametophyte of angiosperms are not a satisfactory index of broader genetic relationships, finds support here in this peculiarity of the structure and development of the perianth and endosperm of *Hedyosmum*.

Other peculiarities of endosperm development, however, such as the complete replacement of nucellar tissue by endosperm, occur more constantly throughout large groups than such features as a single type of tapetum or embryo sac, or of a particular number of potential megaspores. These peculiarities, therefore, may have considerable weight in determining such broader relationships.

Summary

1. The staminate flowers usually occur in long-stalked ovoid catkins which arise in a pair at the base of the cymelike female inflorescence. Each stamen possesses 4 distinct microsporangia.

2. The carpellate flowers occur in sparsely flowered panicles. They have a single pistil, although there is some evidence that 3 carpels enter into the formation of this pistil (5). The perianth of the female flower is initiated before the carpel. It is connected with the surface of the ovary by means of a narrow longitudinal band of tissue which extends along each of its 3 corners; by a similar narrow zone about its base; and by another zone of attachment around the ovary in the apical region just below the base of the style. The perianth persists in the mature fruit and probably constitutes an added protection to the seed.

3. The ovary is 1-celled. Its wall is composed of 15 layers of cells of which the epidermis and the next 3 layers within it are small and but slightly thickened. The cell walls of the fifth, sixth, and seventh layers adjoining the 4 just mentioned are at first unevenly thickened, then, during the ripening of the seed, the entire cavity of each of the cells becomes filled. The inner layer of cells of the ovarian wall next the seed is also considerably thickened in the ripe seed. These 3 thickened layers form the chief protection of the ripe seed.

4. The ovule is pendulous and orthotropic. It bears 2 integuments, the inner being the longer from the outset of development. These are quite thick in the mature seed around the micropylar region, but elsewhere they are unthickened and are scarcely discoverable in the mature seed.

5. A primary archesporium arises from a hypodermal cell of the nucellus, which by dividing produces a tapetal cell and a

definitive archesporial cell. The latter divides into three (usually four) potential megaspores. The lower or chalazal one of these is the functional one, the remaining three degenerate and are absorbed. The mature 7-nucleate embryo sac is of the type most characteristic of angiosperms.

6. The endosperm nucleus, embodying two polar nuclei and possibly a male nucleus, begins to divide before the oospore. Its first division is immediately followed by a transverse wall which divides the embryo sac into upper and lower cells. Each of these two cells continues to divide repeatedly, thus forming the thousands of endosperm cells that completely fill the mature seed except for the embryo. The several layers immediately surrounding the embryo are at this time devoid of starch.

7. The fertilized egg begins to divide only after 15–20 endosperm cells have been formed. The synergids and antipodals degenerate by the time of the first division of the oospore.

8. The ripe seed consists of a globular mass of cells with a poorly developed suspensor. The seed coat is not developed appreciably. Its function is obviously performed by the wall of the ovary.

9. At germination the small embryo is for a long time inclosed and nourished by the swelling endosperm. The cotyledons remain in the endosperm until nearly all its starch is exhausted. They are then withdrawn and assume an active photosynthetic function.

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EXPLANATION OF PLATES XXXIV–XXXVI

All figures are camera drawings from microtome sections except figs. 9, 10, 24, and 25. Abbreviations used: *Ant*, antipodals; *Br*, floral bract; *EdN*, endosperm nucleus; *Em*, embryo; *Esp*, endosperm; *Fl*, flower; *In*, integument; *IIn*, inner integument; *MN*, male nucleus; *MMC*, megaspore mother cell; *OC*, oil containing cell; *OIn*, outer integument; *Osp*, oospore; *OvC*, ovarian cavity; *PC*, perianth cavity; *Pe*, perianth; *Pi*, pits; *Po*, pore; *PT*, pollen tube; *Sg*, synergid; *SyC*, stylar canal; *Tp*, tapetal cell and tapetum; *VB*, vascular bundle; *VB-ov*, *VB-nc*, *VB-pe*, *VB-sy*, vascular bundles of ovary, nucellus, perianth, and style; *WO*, wall of ovary.

FIG. 1.—Transverse section of stamen showing 3-layered wall, tapetum, and young pollen mother cells; ×110.

FIG. 2.—Detailed drawing of archesporium of one microsporangium with tapetum, etc., shown in fig. 1; ×650.

FIG. 3.—Tetrad of young pollen grains; ×1230.

FIG. 4.—Transverse section of nearly mature anther; ×110.

FIG. 5.—Section of mature pollen grain; ×1100.

FIG. 6.—Polar view of wall of mature pollen grain; ×1100.

FIG. 7.—Longitudinal section of young female flower and bracts; ×55.

FIG. 8.—Similar section of two flowers, upper slightly older than one shown in fig. 7; ×55.

FIG. 9.—Surface view on one side of a young female flower; ×55.

FIG. 10.—Surface view of one side of older female flower; ×55.

FIG. 11.—Transverse section of female inflorescence showing various early stages in carpellary development; ×50.

FIG. 12.—Longitudinal section of female inflorescence; ×50.

FIG. 13.—Similar section of very young flower showing initiation of ovarian cavity; ×550.

FIG. 14.—Similar section at later stage showing initiation of style and stigma; ×250.

FIGS. 15–20.—Successive transverse sections (numbered from base to apex) of single young female flower and subtending bract; ×55.

FIG. 21.—Diagram of wax model of vascular bundle system of perianth and ovary.

FIG. 22.—Longitudinal section of flower at time of ripe embryo sac; ×60.

FIG. 23.—Tangential section of one of thickened pitted cells of ovarian wall; ×70.

FIG. 24.—Surface view of young inflorescence showing bracts and two young flowers; ×20.

FIG. 25.—Surface view of ripe fruit; ×20.

FIG. 26.—Nearly longitudinal section of young ovule showing tapetal and definitive archesporial cells; ×650.

FIG. 27.—Similar section showing inner and outer integuments, origin of tapetum, and increase in size of megaspore mother cell; ×650.

FIG. 28.—Similar section of ovule at still later stage showing inner integument closed above nucellus to form micropyle; ×650.

FIG. 29.—Longitudinal section of nucellus showing young embryo sac with 2 degenerating megaspores above it; ×600.

FIG. 30.—Similar section at later stage showing embryo sac and 3 degenerating megaspores; ×600.

FIG. 31.—Similar section of older nucellus showing polarity of 4-nucleate embryo sac; ×600.

FIG. 32.—Longitudinal section of embryo sac showing mitosis in division of 4 nuclei like those shown in fig. 31; ×1100.

FIG. 33.—Similar section of a mature 7-nucleate embryo sac; ×570.

FIG. 34.—Similar section of micropylar half of mature embryo sac showing typical position of egg apparatus and uninucleolate endosperm nucleus; ×570.

FIG. 35.—Similar section of embryo sac after pollen tube has entered; ×570.

FIG. 36.—Longitudinal section of embryo sac showing first two endosperm cells; ×600.

FIG. 37.—Similar section showing further division of endosperm and antipodals still persistent at base of embryo sac; ×600.

FIG. 38.—Similar section of *H. arborescens* at slightly later stage than shown in fig. 37; ×600.

FIG. 39.—Similar section of micropylar portion of embryo sac at 3-celled stage of embryo; ×500.

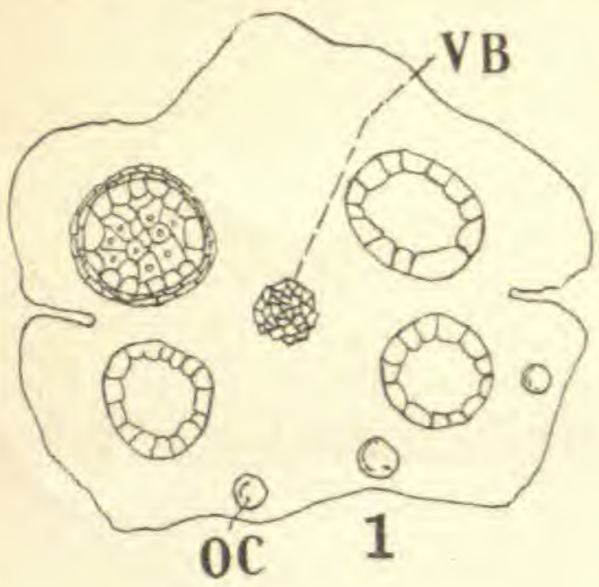
FIG. 40.—Drawing of similar section at later stage showing embryo; ×500.

FIG. 41.—Longitudinal section of micropylar end of nucellus and embryo sac showing character of endosperm around embryo; ×650.

FIG. 42.—Longitudinal section of nearly mature seed showing embryo, endosperm, remains of nucellus, and surrounding wall of ovary with protective cells and still persistent perianth; ×60.

FIG. 43.—Similar section of nearly ripe seed showing embryo, endosperm, and one integument at micropylar end; ×125.

FIG. 44.—Longitudinal section of germinating seed removed from fruit showing absorbent cotyledons still imbedded in endosperm; ×60.



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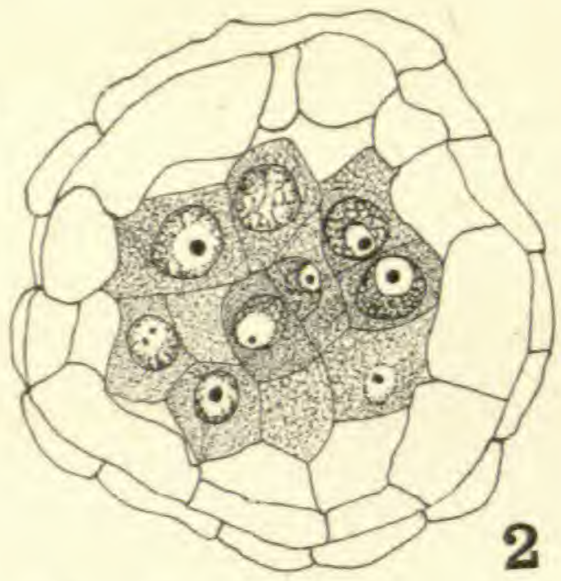

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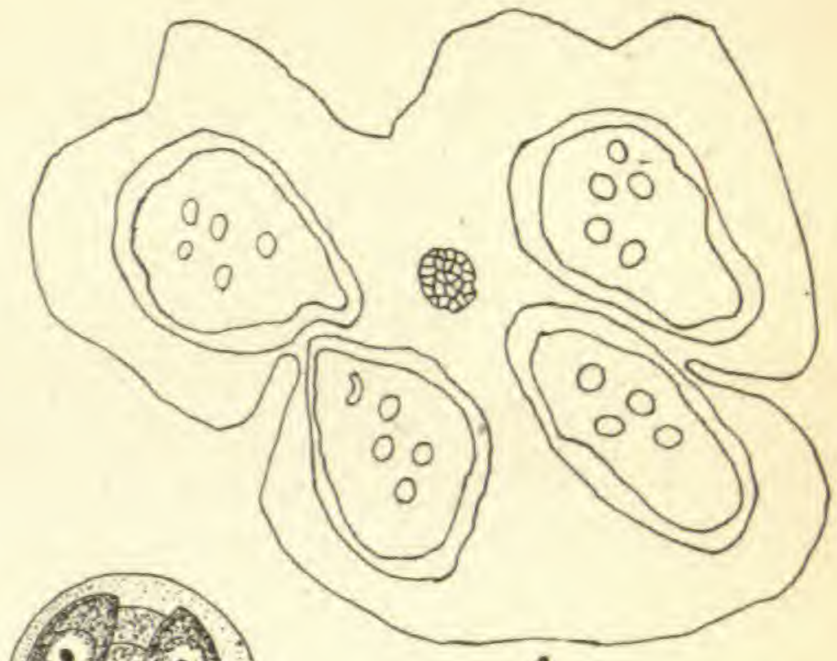

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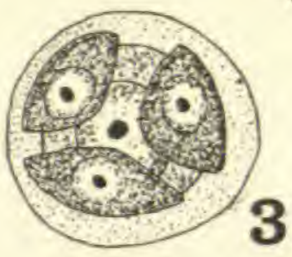

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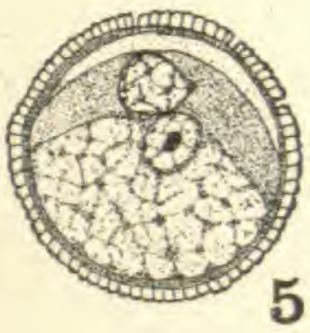

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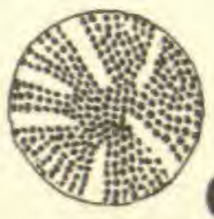

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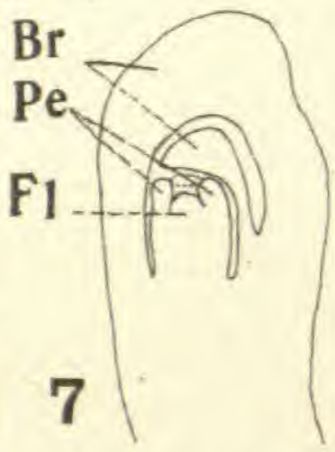

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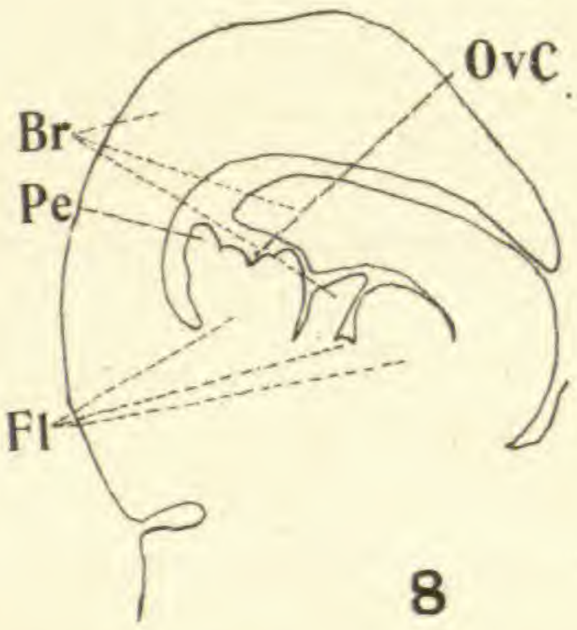

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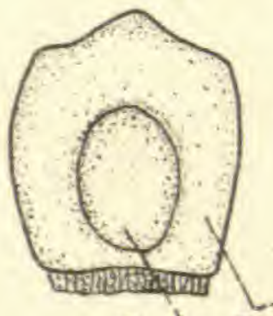

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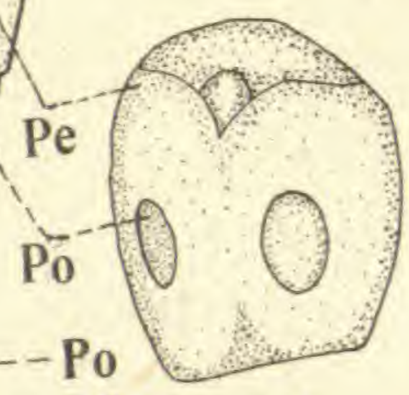

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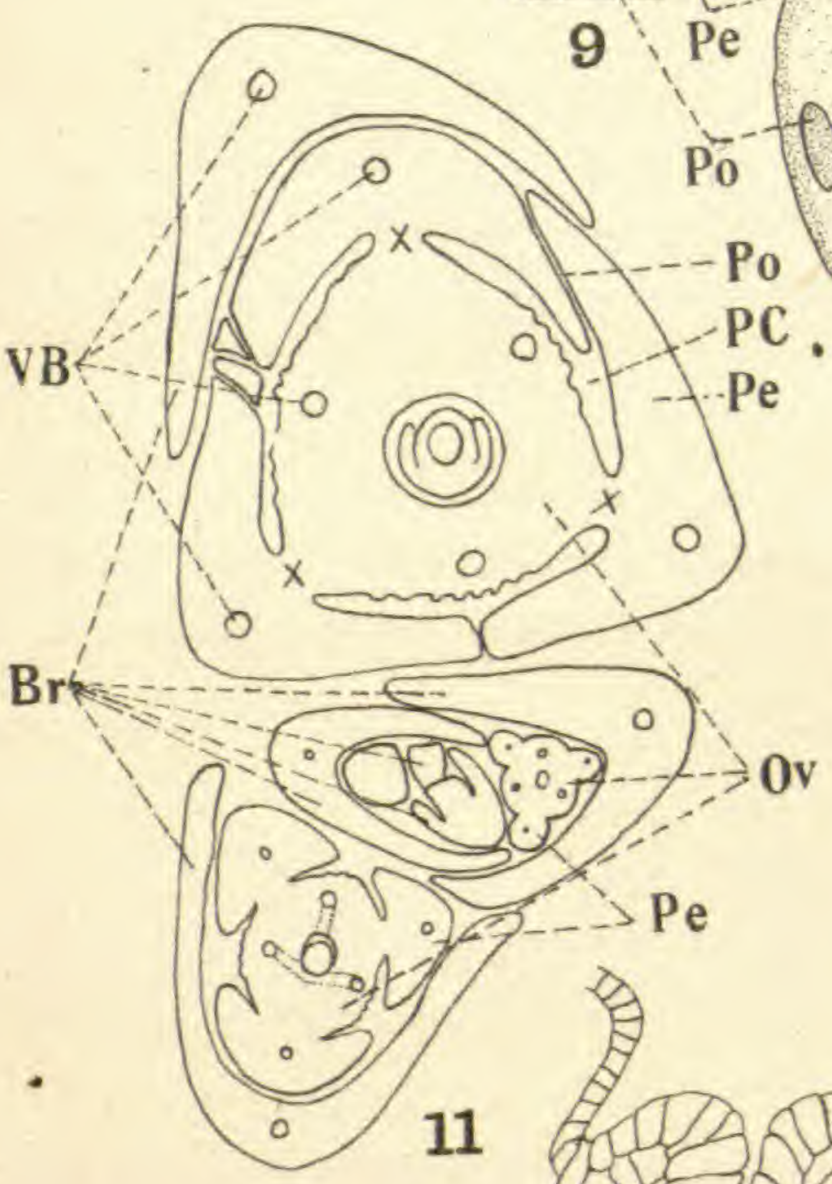

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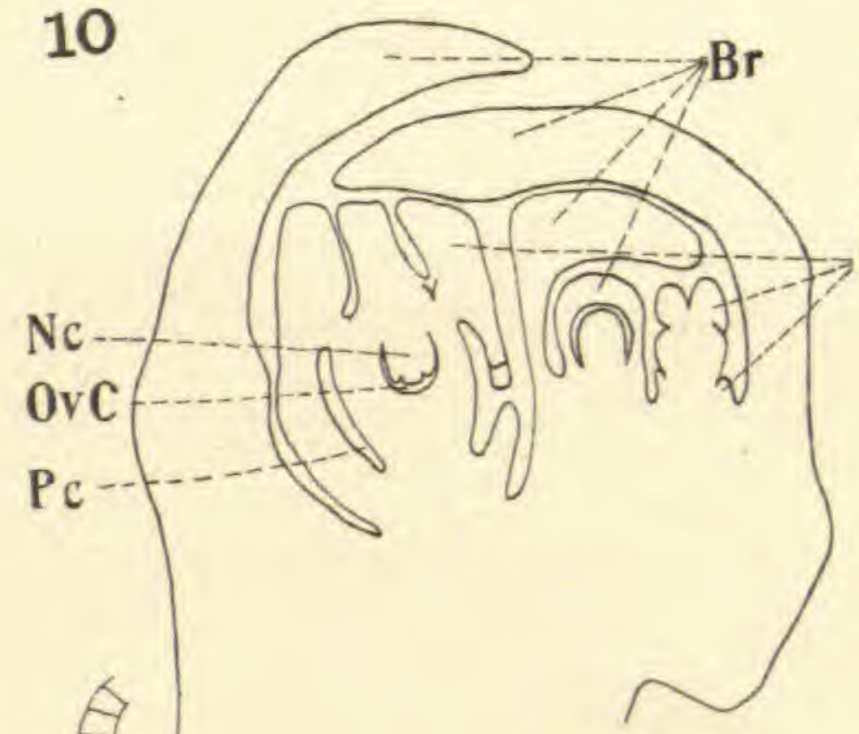

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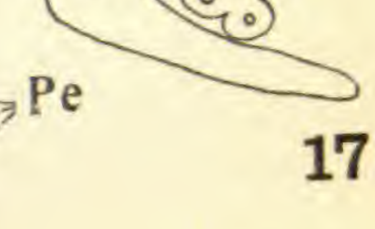

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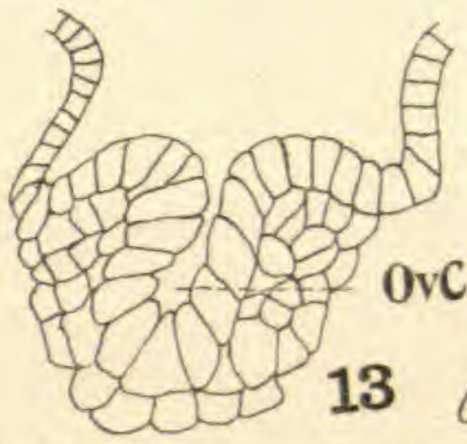

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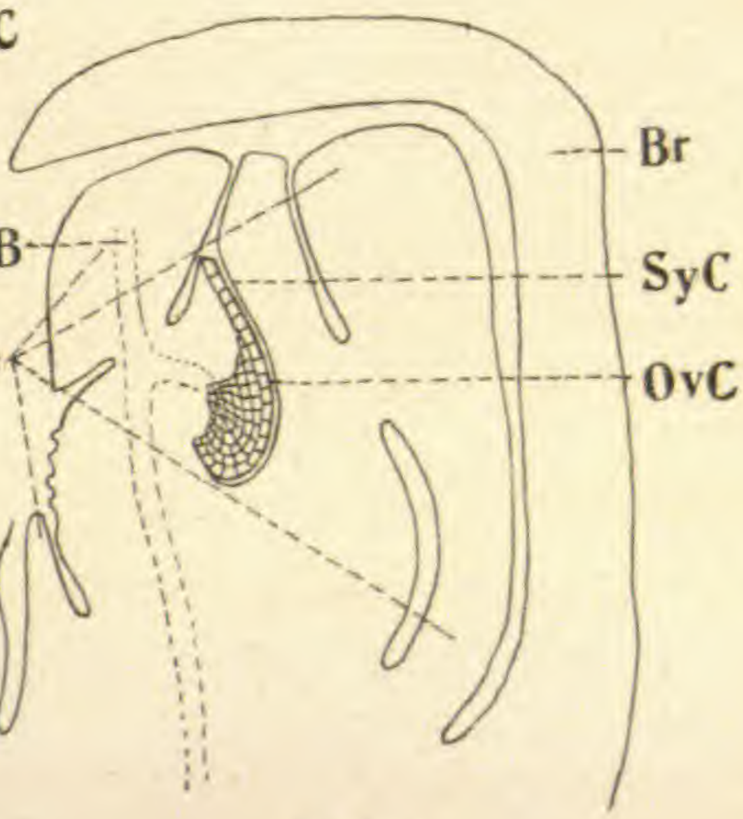

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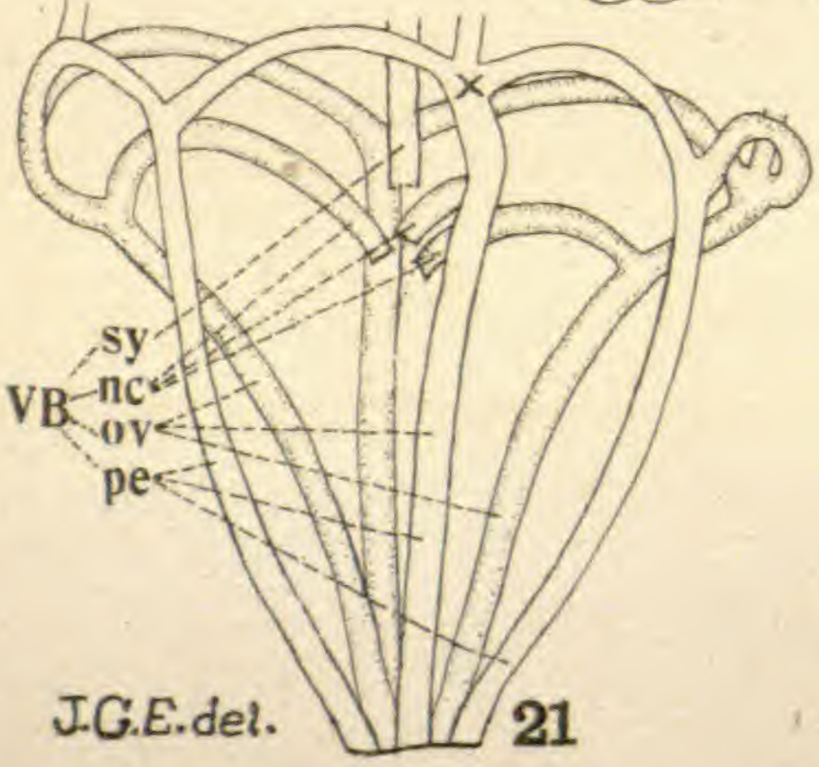

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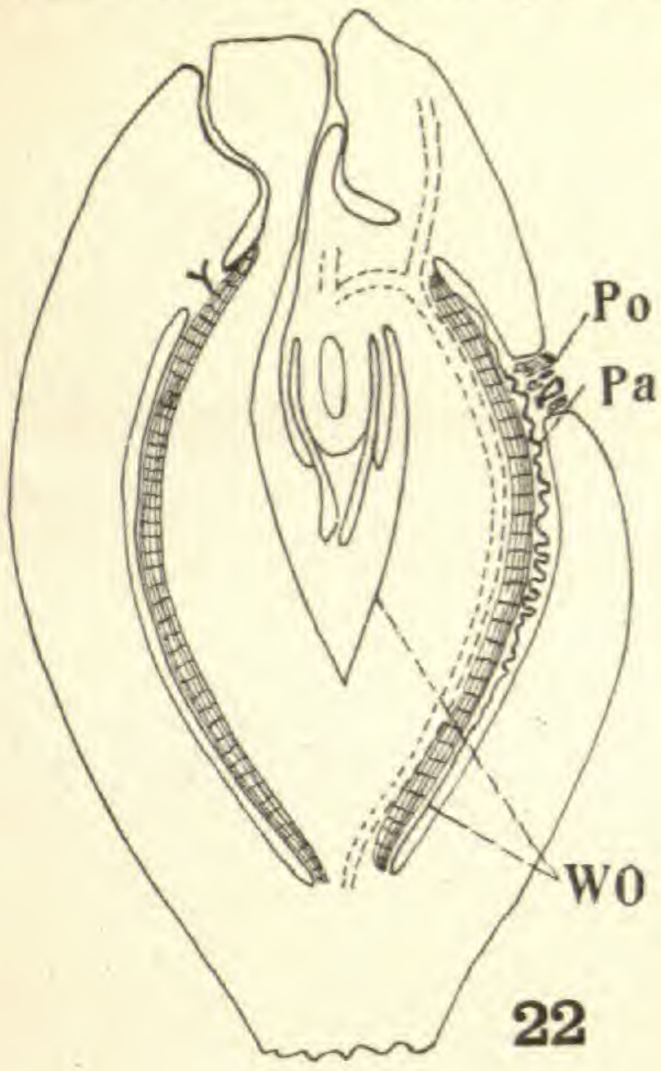

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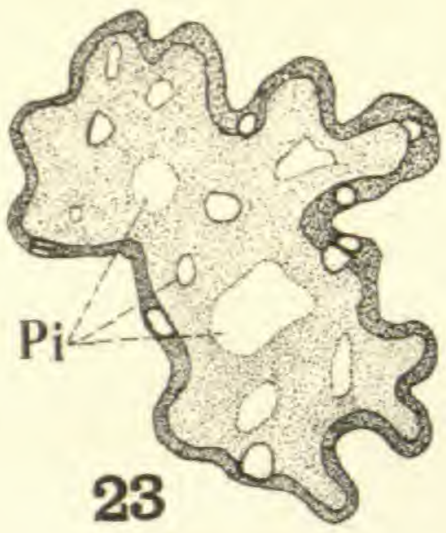

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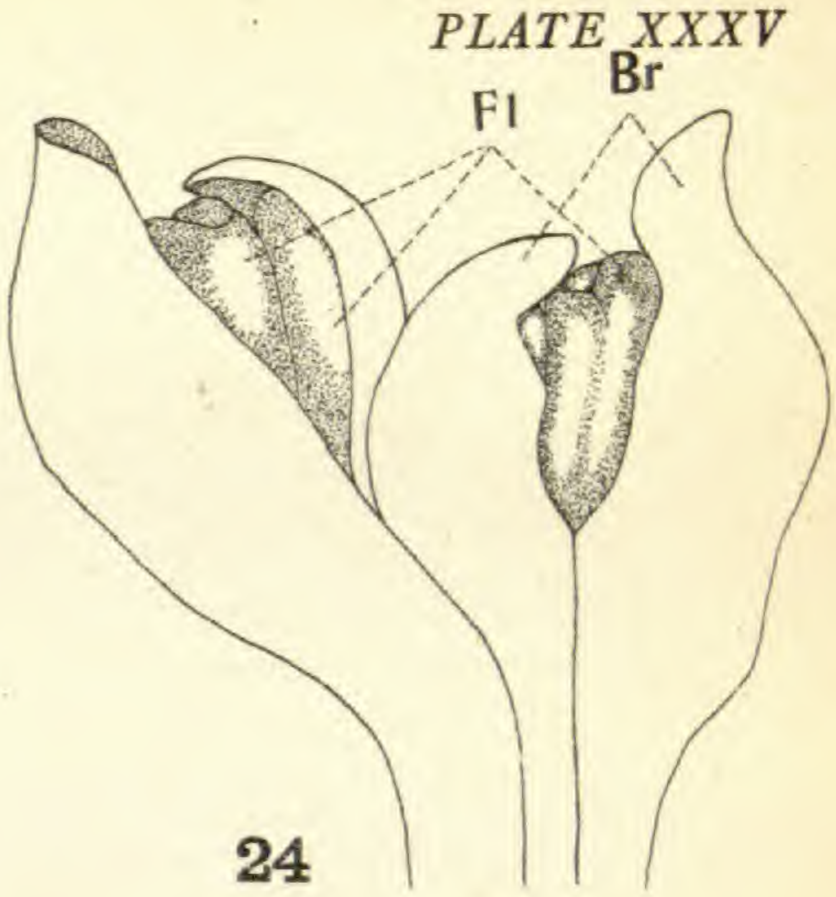

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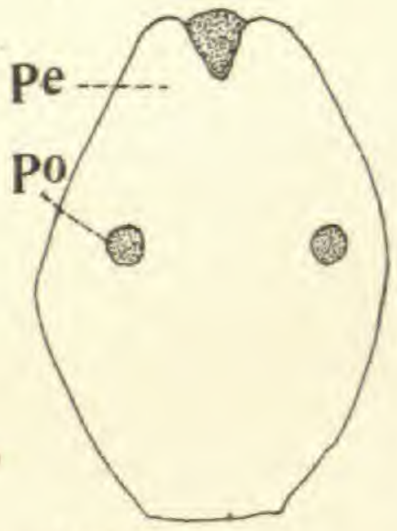

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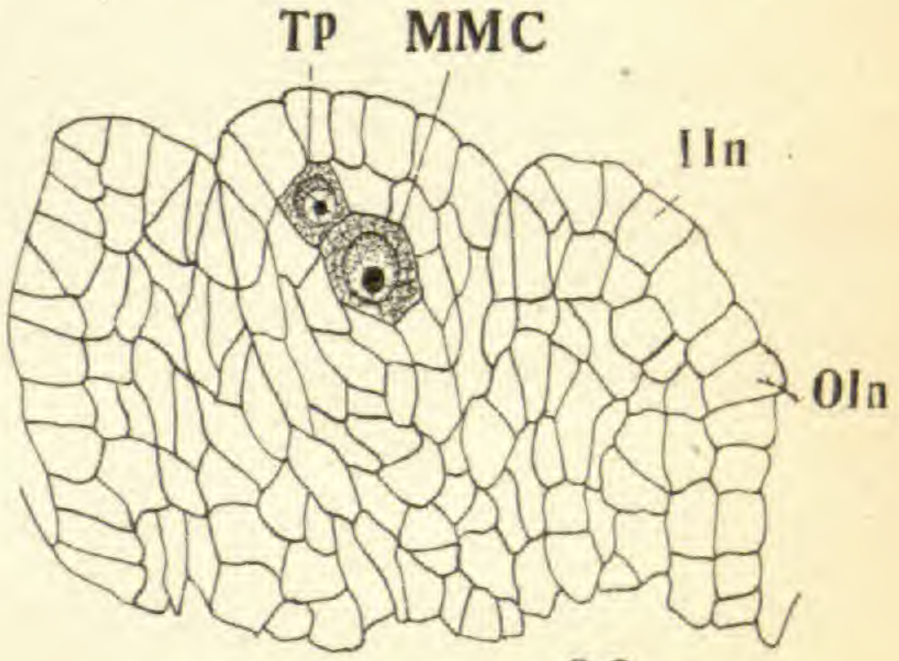

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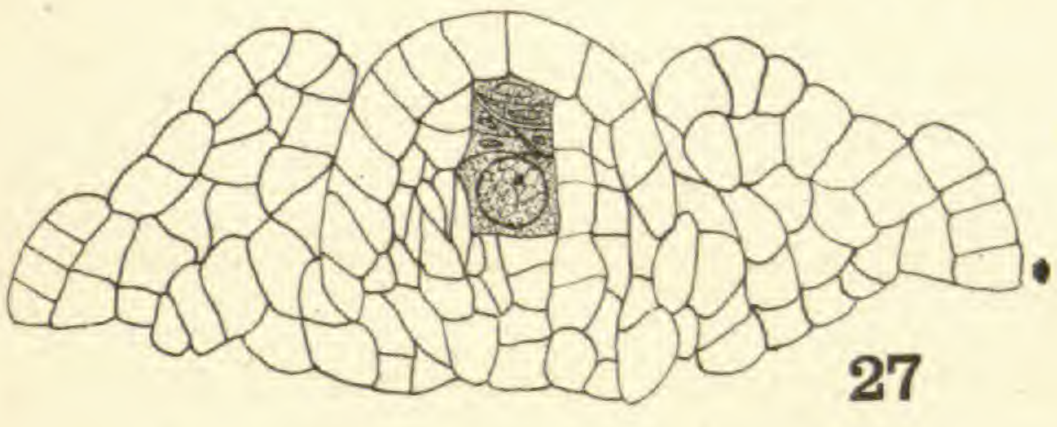

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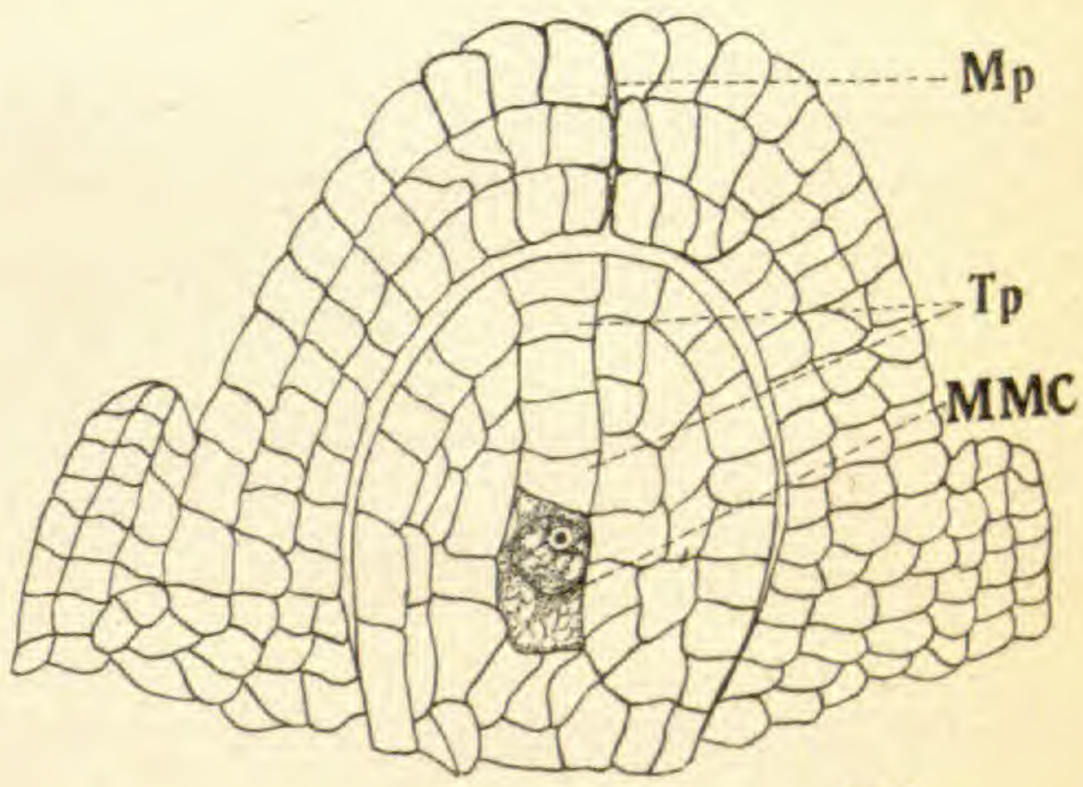

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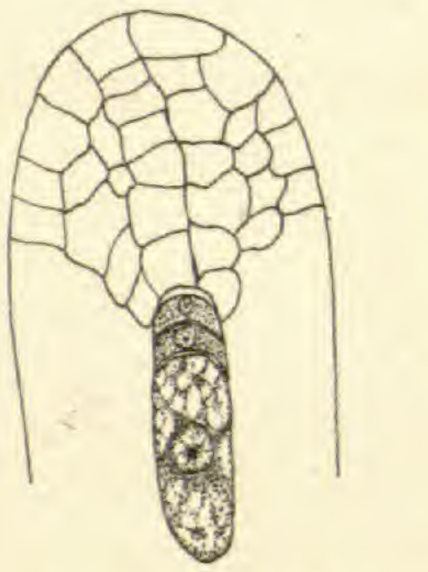

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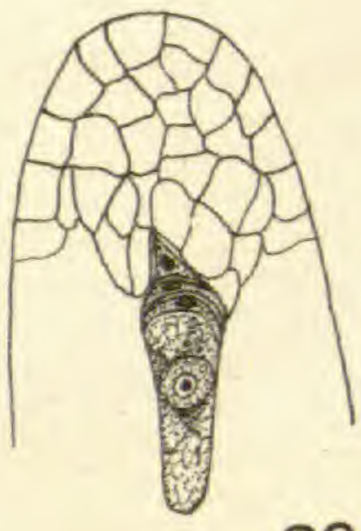

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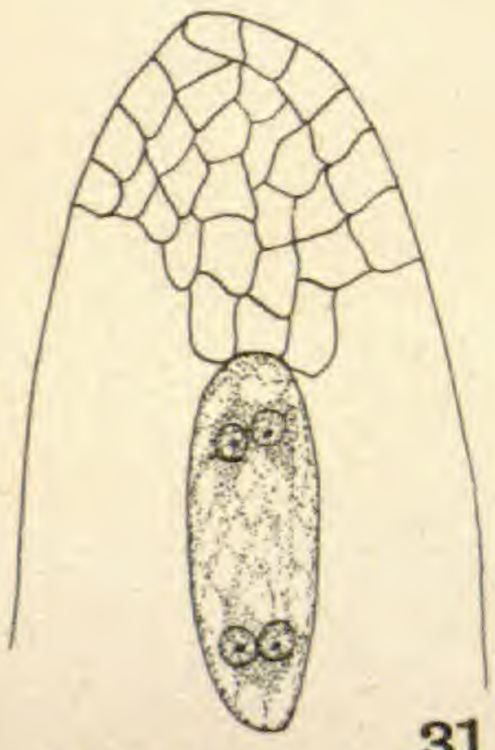

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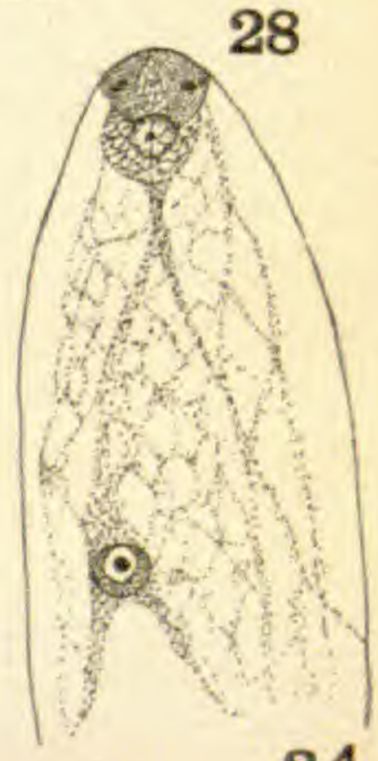

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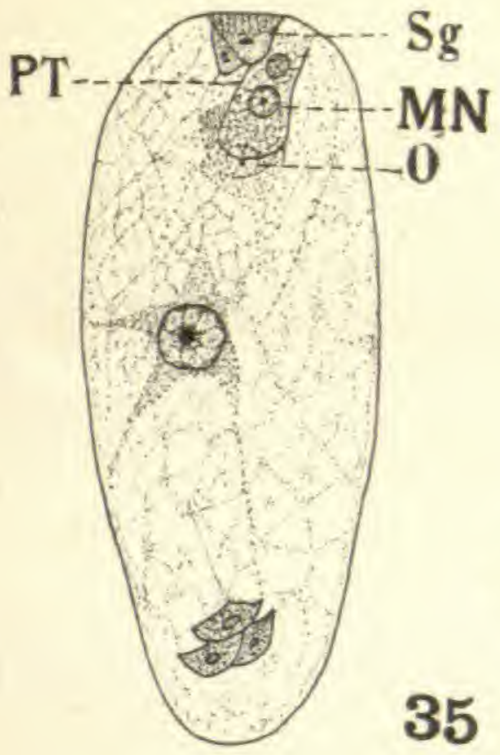

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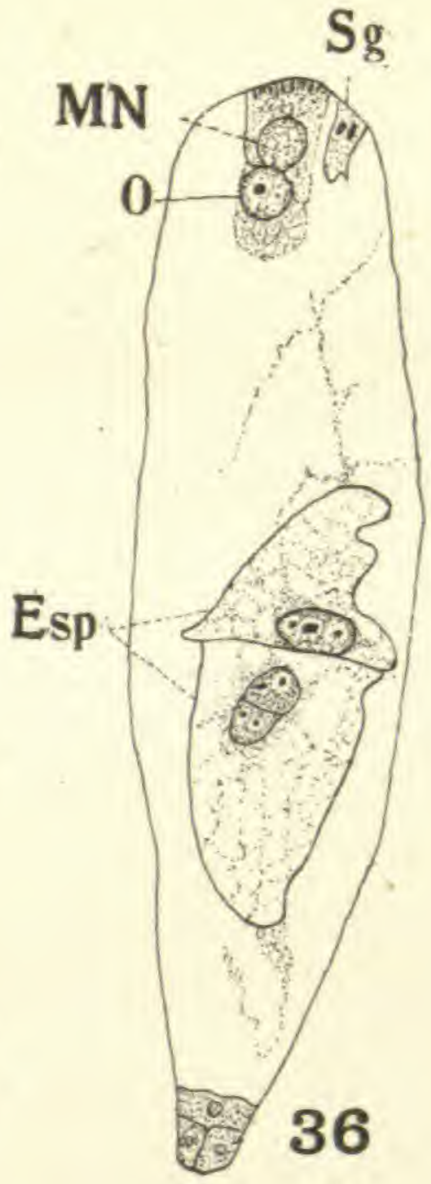

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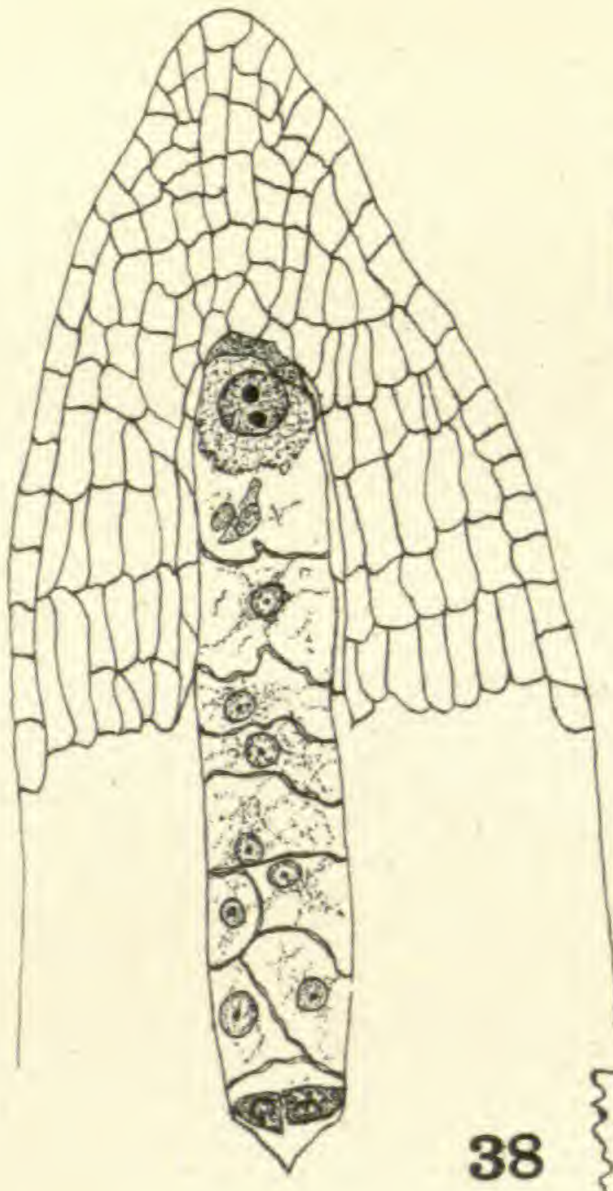

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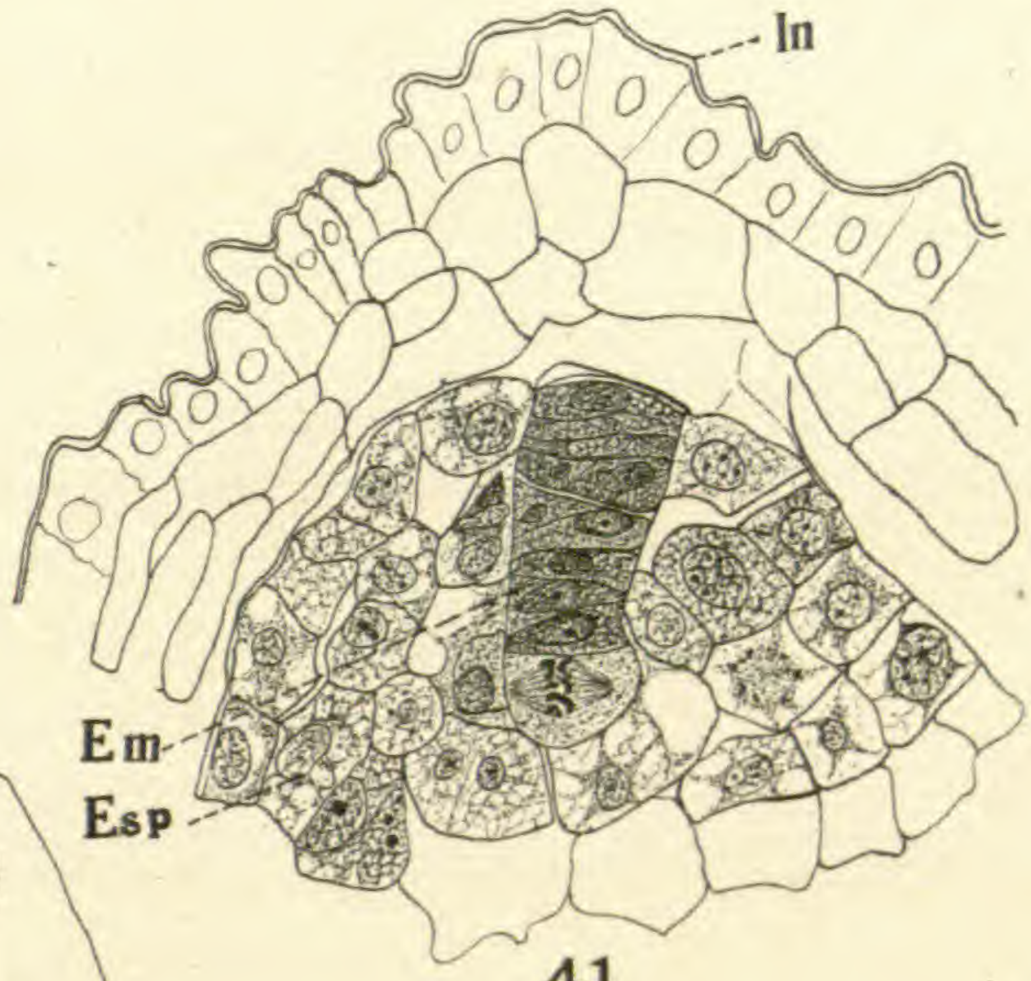

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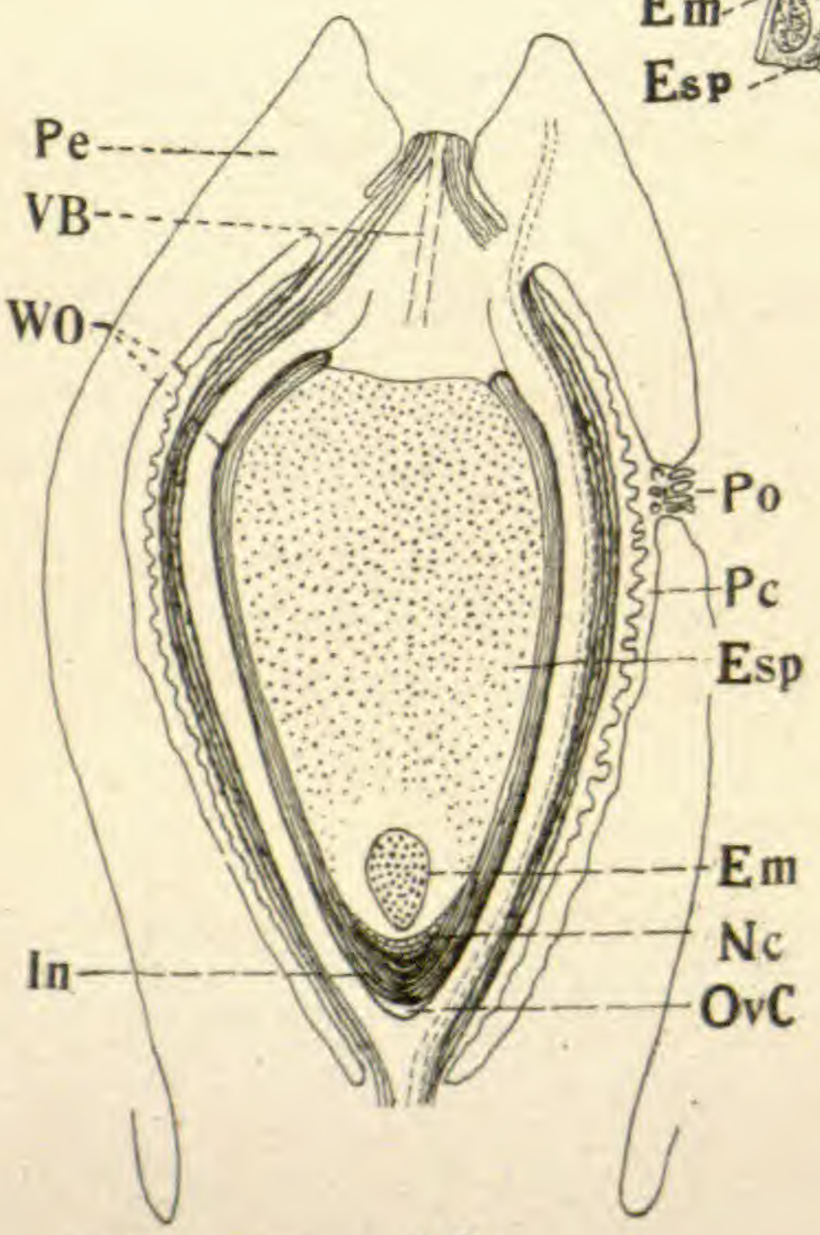

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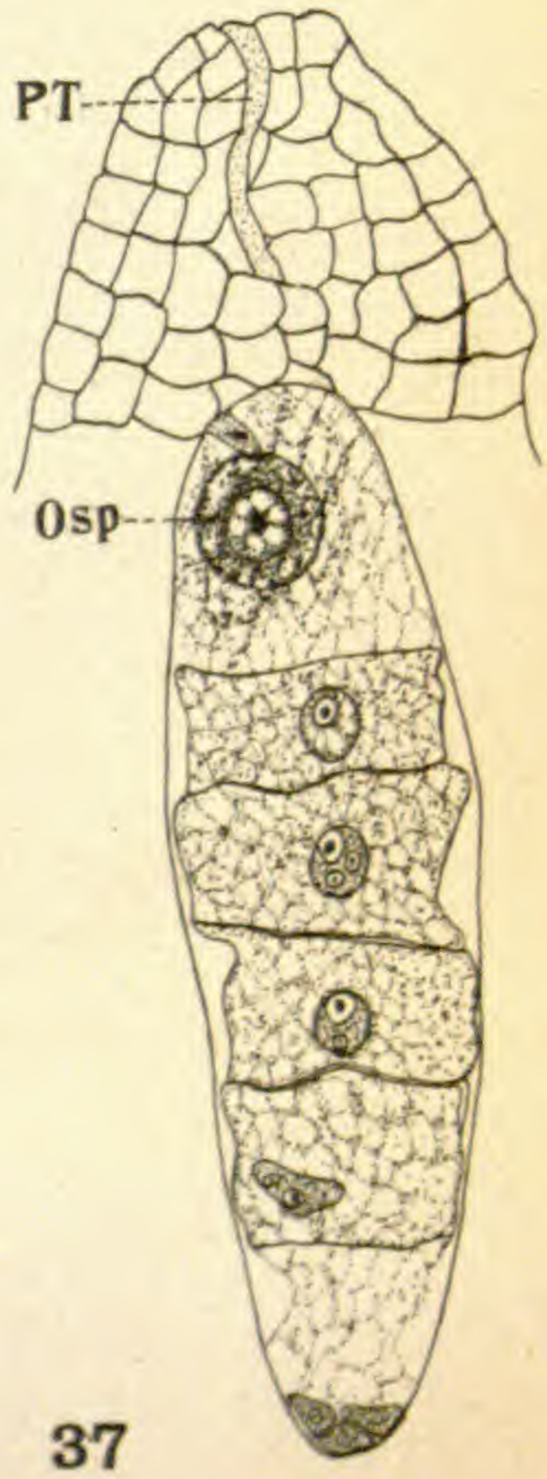

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J.G.E. del.

SOME CHARACTERS OF XYLEM TISSUE IN CYCADS.

H. B. SIFTON

(WITH PLATES XXXVII, XXXVIII AND ONE FIGURE)

The detailed investigation of certain anatomical features of the Cycads has been undertaken in the hope of throwing light on the origin of the more specialized structures occurring in the higher Gymnosperms. In recent years considerable work has been devoted to determining the details of anatomical structure in the Conifers. These details have been given much prominence as evidences of the inter-relationships of the various groups. A lack of knowledge of the ancestry of the structures themselves, however, has minimized their value as criteria in phylogenetic investigations. This knowledge can be supplied only by a study of primitive forms.

Pitting

The shape and arrangement of bordered pits in the woody tissue have long been regarded as valuable phylogenetic data. It is largely owing to these features that the Araucarians have been supposed to be closely related to Cordaitean forms, and many botanists still hold this view, notwithstanding the arguments advanced by JEFFREY (6) and SEWARD (9) in favor of different lines of ancestry for the family. In 1907 GOTHAN (4) worked out a phylogenetic line of development of bordered pitting, considering the most primitive type to be hexagonal and crowded over the whole tracheid wall. According to his theory, the pitting next became eliminated from the tangential walls, but still covered the radial as before. A further elimination resulted first in small isolated groups of pits, then in the uniseriate flattened condition, and finally in the scattered arrangement, where the pits occur singly on the tracheid wall. This series of eliminations took place on the middle part of the wall of the tracheid, the crowded arrangement being retained on the ends to facilitate vertical movement of the sap. GOTHAN found his types of arrangement combined in a fossil plant, but in no living form.

JEFFREY is more conservative than many others in his estimate of the significance of pitting, but considers that it is of distinct value in classification when its character in all parts of the plant is considered. On this ground, in his work on the Araucarineae, he accepts the presence of opposition pitting in the cone axis, and scattered pits in the seedlings, as denoting descent from an Abietineous type. He has neglected the character of the pitting in primitive forms such as the Cycads, however, and his interpretation is not in harmony with the facts which these forms disclose. This was done, notwithstanding the fact that as early as 1840 DON (2) recognized the value in phylogeny of the study of Cycads. He carefully worked over the character of tracheids by such methods as were in vogue at that time, and agreed with MEYENS, a still earlier investigator, that the spiral, scalariform, reticulate, and border-pitted types could be referred to a common origin. The importance of these transitions was emphasized also by PENHALLOW (7) in 1907 as affording valuable data on the ancestral character of the bordered pit of the higher forms. In 1919 BAILEY (1) argued that opposite pitting is formed by the breaking up of bordered scalariforms, and that the alternate type was formed from this by a "staggering" of the rows of pits.

In this paper certain features of the primary wood of the Cycads will be considered first. Fig. 1 is a longitudinal section of the petiole of *Cycas revoluta*, showing the tangential walls of the tracheids in the neighborhood of the protoxylem. The tracheid *a* shows the characteristic spiral and scalariform structure of the protoxylem. In transverse section (not figured) the scalariform bars are seen to arch over the intervening spaces so as to form very narrow borders. On the cell *b* the scalariforms are more closely approximated, and through the slits may be seen shorter pores, belonging to the adjacent wall of the next tracheid. The tracheid *c* also shows this clearly. In the other two tracheids typical bordered pits are present. The type of scalariform from which such bordered pits are formed is shown in fig. 2. It is a scalariform similar to that formerly described, except that the borders are wider. Fig. 3, another section from a *Cycas* petiole, indicates transitions in the formation of ordinary bordered pits from this

type of scalariform. Below the center of the figure is a scalariform reaching from side to side. Its border shows constrictions at two points, evidently the beginning of a division into three bordered pits. In the portions of the tracheid above and below, complete divisions and other incomplete ones are in evidence. The name "fusion pits," which has been applied to similar appearances, is evidently a misnomer in this case. They plainly represent phases in the breaking up of the ancestral scalariform rather than the union of two of the more specialized bordered pits. The small pits on tracheids *a* and *b* of fig. 1 in all probability are formed from the narrow bordered scalariforms in a similar manner.

Figure 4 is a much lower magnification of a longitudinal radial section of the fossil stem of *Lyginodendron Oldhamium*, acknowledged to be one of the most ancient of the seed plants. This form had attained in the secondary wood of its stem the condition represented in GOTHAN's second type, the pits being practically eliminated from the tangential walls (cf. SCOTT 8), but crowding the radial walls from end to end of the tracheid. Wherever the cell wall is present in the figure it is seen to be completely covered with the type of pitting known as recticulate. A careful examination of the pits shows them to be of the same type as those in fig. 1, which had their origin in the narrow bordered scalariforms.

The stem of *Cordaites* (fig. 5) has pits which, like those of the Cycads, have probably originated from the cutting up of wide bordered scalariforms, a condition quite in keeping with the general higher type of wood structure exhibited in the Cordaitean forms.

Further evidence of the origin of the bordered pit from the scalariform is found in the secondary wood of certain of the Cycads. A type of fusion pit which recalls the condition in the narrow bordered scalariforms of fig. 1 is shown in fig. 10, which is a radial section of the stem of *Dioon spinulosum*. The three pits nearest the top are of the short, slightly flattened type quite common in these forms. The next three are more elongated. All show the characteristic cross pores of adjacent elements. The seventh of the series is a pit of the second fusion type. It appears like two pits, each with a short pore, with a common long pore crossing both.

On one tracheid wall (in this case the one beneath) two separate pits have formed, each with its own pore; while on the adjacent wall of the next tracheid, one large, somewhat scalariform pit has been retained. Just below this comes a pair of completely separate pits, from their shape and approximation evidently formed by the division of what potentially was a single long one. Then come two more pits of the fusion type, after which the regular type of pitting is resumed. Such examples are often found at the ends of tracheids, where, as noted later, there are other primitive characters.

The multiseriate condition is the most common arrangement of pits in Cycads. In some cases the pits are so closely approximate as to appear slightly flattened. This is the typical condition in the Cordaiteae as described by SCOTT, and has generally been considered the most primitive bordered condition, although more specialized than the reticulate type. The outline of the pits in the Cycads, however, is more often curved.

In the Cycads the scattered type of pitting is also found, originating by the elimination of pits from portions of the tracheid. Fig. 6 from a radial section of a *Dioon spinulosum* stem shows this feature. In the lower part of the right hand tracheid we have biseriate pitting covering the radial wall, with here and there a pit obliterated. The position of the vanished pits is indicated by lighter areas caused by the thinning of the primary wall. These are the primordial pits of Sanio, which have formed as if bordered pits were to be located over them as usual. Farther toward the top is a single row of somewhat flattened pits, an arrangement common in the Araucarians. Still farther up some of the pits have become smaller, while others have been eliminated entirely, thinning of the primary wall being visible here and there. This scattered pitting is seen also in other tracheids of the figure, the primary pit areas being very evident, especially just above and below the center of the second tracheid from the left. At the center is an interesting small bordered pit surrounded by a slightly larger area, probably the boundary of the primordial pit. This seems like a case of partial elimination. The irregular obliteration of the pits has left in some places isolated groups of pits like those

referred to by GOTHAN. A further eccentricity of the elimination is illustrated in the tracheid to the right of fig. 11. The lighter colored pits have typical bilateral borders, while the obscure ones are unilateral, the corresponding pit on the overlying tracheid not having been formed.

The arrangement of pits, opposite or alternate, deserves notice. In fig. 6 two or three pits occur in a horizontal row. In some cases as many as four such pits have been found. In the more common condition, not figured, the pits are regularly alternate, sometimes round, and sometimes flattened by mutual contact. Just as the scalariforms of one tracheid are horizontal while those of the next are inclined, and either straight or curved and irregular (fig. 1), so on one tracheid is found the opposite arrangement of pits, and adjacent to it the alternate. There seems no reason for believing that alternate pitting is formed by any disarrangement of rows of round bordered pits. More specialized plants have one or other of these types predominating; for example, the alternate and flattened arrangement in Araucarineae, and the scattered, grouped, and opposite in the Abietineae. The presence of all these conditions in mature Cycad wood, as well as in the ancient fossil form described by GOTHAN, modifies to a great extent their phylogenetic significance in higher forms. It strengthens BAILEY'S statement that the presence of opposite pitting as well as alternate in the cone axes of Araucarians cannot properly be used as an argument for their descent from the Abietineae, and neither is the same condition in primitive parts of pines an evidence of descent from an Araucarian type. It would seem that if these facts have any significance in phylogeny, they indicate that both pines and Araucarians are descended from lower forms which contained both these arrangements.

It may be stated in passing that both opposite and alternate arrangements of pitting occur in the Cordaiteae, the alternate, however, being greatly predominant. Instances of opposition pitting in Cordaites may be seen in fig. 5, in the upper part of the right hand pitted tracheid. There is an example also near the lower end of the second tracheid to the left of it. Instances of GOTHAN'S grouped arrangement are also present, especially in the

lower central part of the figure. The uniseriate and scattered arrangements occur, being formed probably by the suppression of pits, as in the Cycads. The only evidence of this in the fossil section, however, is the decreased size of many of the pits in the region of elimination. A careful examination of fig. 5 will make this point clear.

It will be seen from the foregoing account that the Cycads, besides giving indication of the mode of formation of the bordered pits from the scalariform type, afford valuable data on the interpretation of the arrangement of pits. The elimination in these low

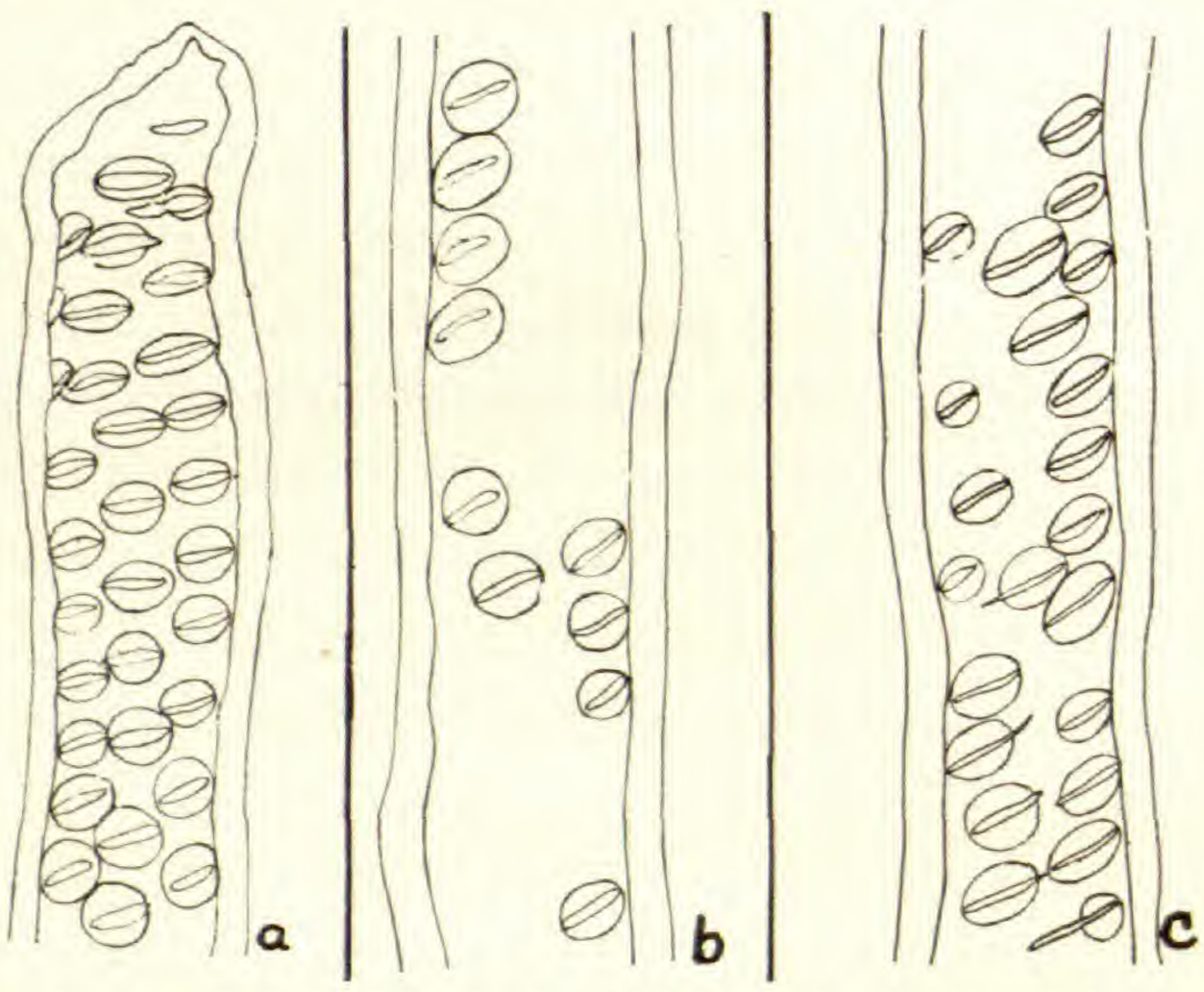

FIG. 1.—*Cycas revoluta:* radial views of different regions of tracheid from stem; *a*, end of tracheid; *b*, normal pitting at contact of two tracheids; *c*, pitting in contact with medullary ray.

forms shows no indication of following a definite law, but proceeds promiscuously, giving rise to all the various types of pitting. It is practically restricted, however, to the middle part of the tracheid, the terminal portions and those parts in contact with the ray cells remaining multiseriate.

The terminal and ray pitting of the tracheids has always remained primitive in another respect. This is indicated in text-fig. 1, which is from a tracheid of the stem of *Cycas revoluta*. In this figure *a* represents the terminal pitting, *b* the ordinary pitting, and *c* the ray pitting of the same tracheid. The pits and their pores are longer in *a* and *c* than in *b*. The pores often extend

beyond the borders in *a* and *c*, and thus recall the condition where pits are forming on scalariform elements (fig. 3). Many of the pores were measured for definite comparison, and the tracheid figured does not exaggerate the difference in pit and pore lengths. Ray pitting on *Dioon spinulosum* secondary stem wood is shown in fig. 9. Here also the multiseriate pitting is present, accompanied by the elongated pore. The left hand tracheid of fig. 11 shows the same type of pore, where a vertical parenchyma cell is in contact with a tracheid. Evidently the primitive type of pore occurs wherever a tracheid is in contact with any type of parenchyma cell. Similar primitive features have been recorded by THOMSON (11) in Araucarian ray pitting.

Tertiary thickenings are common on the tracheid walls, taking the form of spirals or scalariform bars with long shallow pits between. They occur whether bordered pits are present or not, and often traverse the region of the border itself, but have never been observed to cross the pores. PENHALLOW regarded such thickenings as relics of the ancestral manner of deposition of the cell wall, a view which is strengthened by their presence in these low forms.

Bars or rims of Sanio

Considerable importance was attached for some years to the presence or absence of "bars" or "rims" of Sanio. Miss GERRY (3) in 1910 showed them to be present in all families of the Conifers except the Araucarians, and made this a distinguishing feature between both fossil and living Araucarians and other coniferous forms. JEFFREY (6) and THOMSON (11), however, in practically simultaneous publications described bars from the transitional region of the pitting in the cone axis of an Araucarian. This JEFFREY interpreted as evidence of the derivation of Araucarians from the Abietineae. He recognized that this evidence would be invalid if all primitive types of pitting had bars of Sanio, and looked for them in primitive regions of *Cycas* but failed to find them. Their presence here was described later by the writer (10), and invalidates his conclusions. JEFFREY'S misstatement has no doubt been responsible for the exaggeration of the importance of

the structure, and probably led to the rejection of all other criteria, making the bar "an infallible test for tribal affinities" (HOLDEN 5), both in fossil and living Conifers.

In 1919 BAILEY (1) studied the origin and development of bars of Sanio, and concluded that those in transition regions are merely normal middle lamellae left between thinned pit areas in the primary wall. He states that when the pits are opposite the bars go smoothly from side to side of the tracheid, because the pits are formed on one primary scalariform pit area, and the bar is the thickening of the lamellae between this area and the next. This theory, however, will not explain the bars figured in the writer's paper of 1915. Those shown in *Araucaria* are connected with pits in regular horizontal rows, but still fork, following round the circumference of each pit, so as to leave clear diamond-shaped areas bounded by opposing forks. These small areas cannot be considered to be other primary pits. The same fact holds for the bars in the *Cycas* petiole, which fork, and are even split into two separate rims in some cases, although the pits are not far enough apart to make it possible to attribute the thin space to another primary pit area.

Bars of Sanio have now been found in other portions of Cycads than the transitional primary xylem. Figs. 6 and 7 illustrate them in the stem wood of *Dioon spinulosum*. These bars often extend beyond the margins of the pits with which they are in contact, as in the middle tracheid of fig. 7 near the bottom, and so are of a higher type than those figured in the former paper. They are still much more primitive than those of the Abietineae, however, lying in close contact with the pits, whenever such are present. Fig. 6 shows the ordinary type of bar in this plant. Between the pits of the single row on the right are bars of the regular Araucarian type. Their length is not greater than the borders to which they cling, and they spread slightly at the ends. The pitting of this tracheid is conspicuously of the opposite type, so that if BAILEY'S theory of the origin of the bars is entirely correct, they should in this case pass beyond the pits to the limit of the tracheids. In the two left-hand tracheids of fig. 6 is shown a condition which is quite common, namely, the presence of these bars in connection

with primordial pits from which the secondary pitting has been eliminated.

Trabeculae

These spool-shaped bars, extending in radial series across the lumens of adjacent tracheids, have received considerable notice in literature, owing to the confusion which arose in some cases between them and bars of Sanio. They have not before been figured in the Cycads, but are present, as shown in fig. 8, a radial section of *Dioon spinulosum* stem. They contain a core or axis composed of a substance which stains in the same way as the middle lamella of the cell. This core pierces the tangential secondary walls of the tracheid and joins up with the middle lamella. These structures are present in higher forms, but their significance is not known. Since they connect with the primary wall, they must have been laid down before the beginning of secondary thickening.

Summary

1. A study of the primary and secondary wood of Cycads indicates the development of reticulate, alternate, and opposite pitting directly from scalariform types.

2. The grouped, uniseriate, and scattered pitting characteristic of higher forms is shown to be formed by the elimination of pits. In low forms, of which the Cycads are a type, this elimination proceeded without apparent order, forming all types of grouping indiscriminately.

3. Similar arrangements of pits occur in Cordaites, although its type has become more fixed than is the case in the Cycads.

4. The Cycads, like the Araucarians, have more primitive types of pitting at the ends of tracheids and where they come in contact with parenchyma.

5. The xylem of certain of the Cycads quite commonly exhibits spiral tertiary thickenings.

6. Bars of Sanio of the Araucarian type are found in both primary and secondary Cycad wood. An elongated type of bar is also present. The Araucarian type is considered the most primitive in living seed plants. No explanation of its origin is offered by BAILEY'S theory.

7. Trabeculae are present.

This work was done with the advice of Professor R. B. THOMSON. I am indebted to him, not only for advice and encouragement, but also for the supply of materials necessary for the work.

UNIVERSITY OF TORONTO

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DESCRIPTION OF PLATES XXXVII, XXXVIII

PLATE XXXVII

FIG. 1.—*Cycas revoluta:* petiole; tangential section of primary wood; ×225.

FIG. 2.—*Zamia integrifolia:* petiole; pitting of primary wood; ×445.

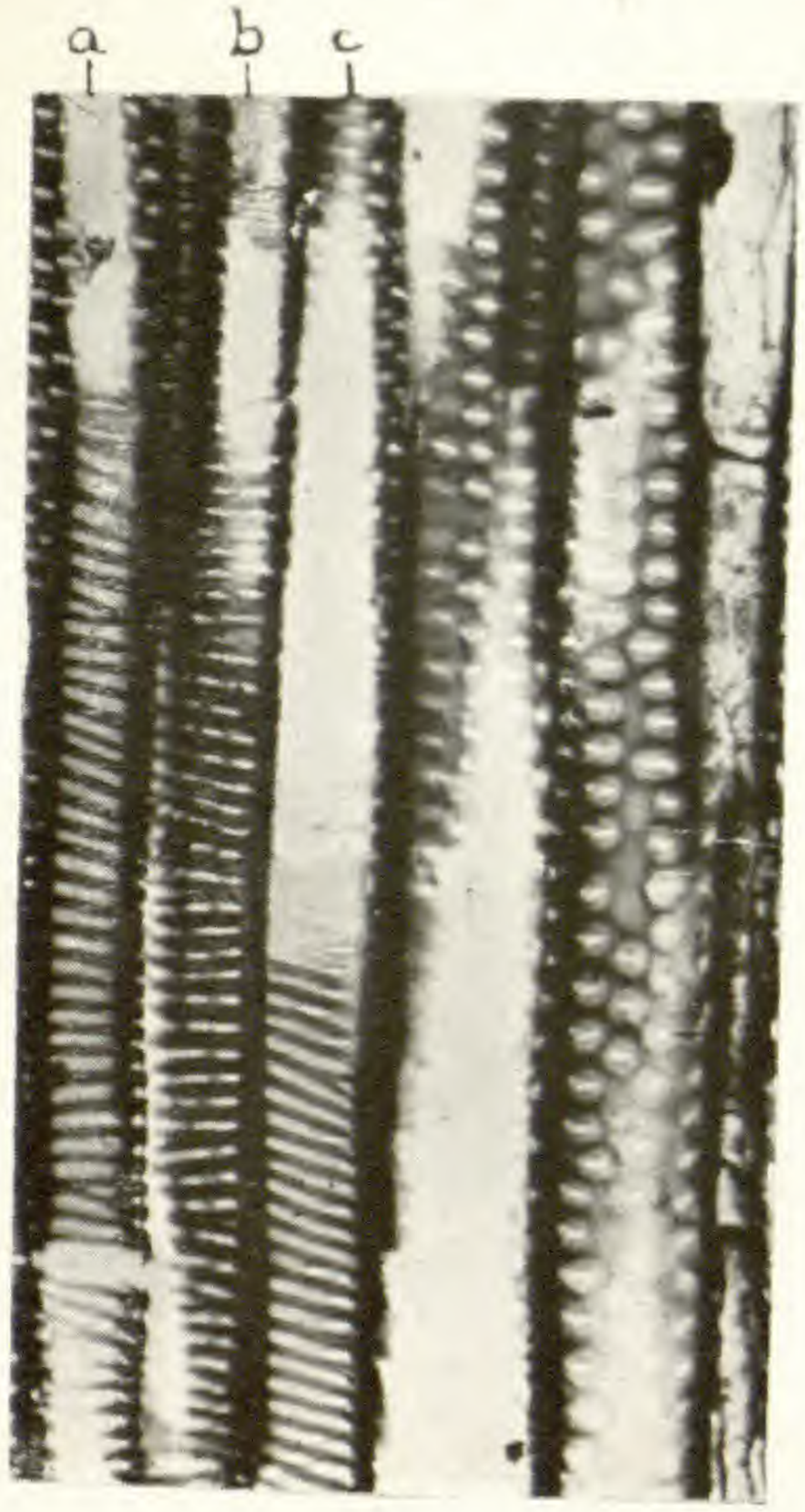
FIG. 3.—*Cycas revoluta:* petiole; primary wood, showing transitional pitting; ×445.

FIG. 4.—*Lygenodendron Oldhamium:* radial section of secondary wood of stem; ×100.

FIG. 5.—*Cordaites* sp.: radial section of secondary wood of stem; ×225.

PLATE XXXVIII

FIG. 6.—*Dioon spinulosum:* radial section of secondary wood of stem, showing pit arrangement and bars of Sanio; ×225.



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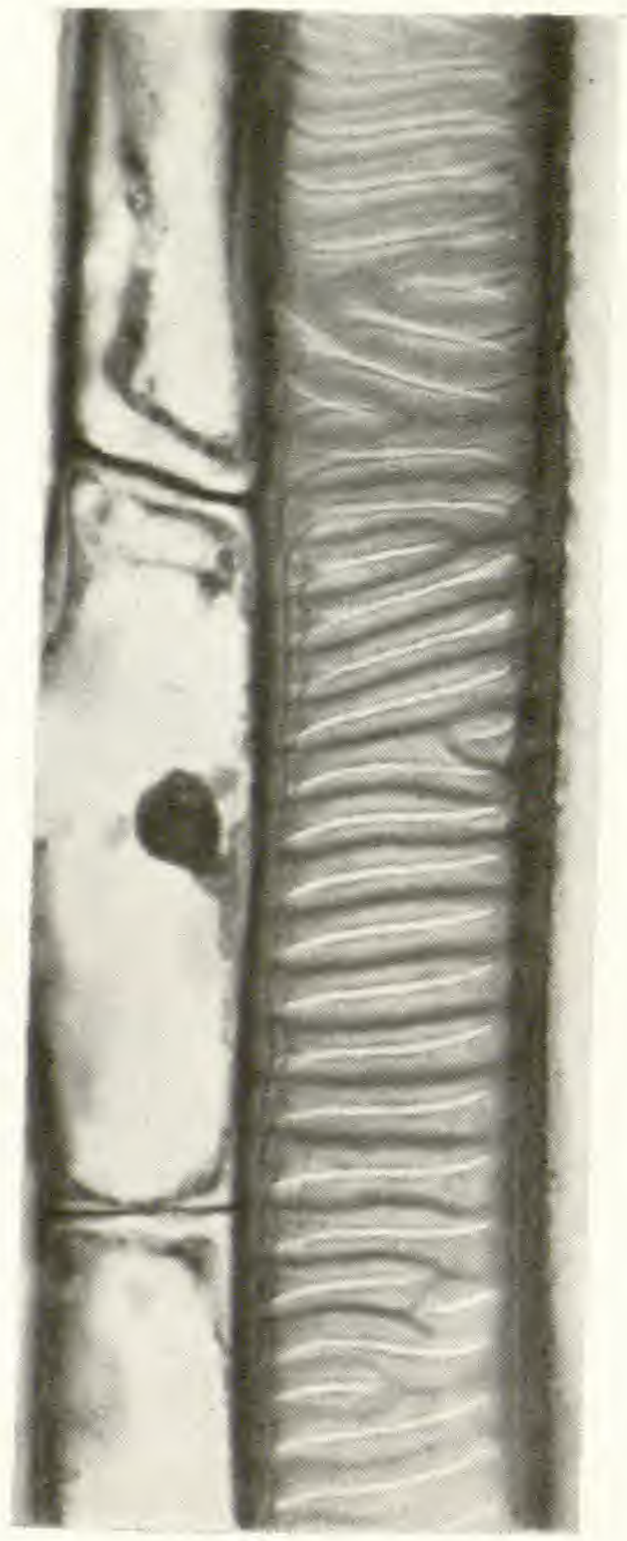

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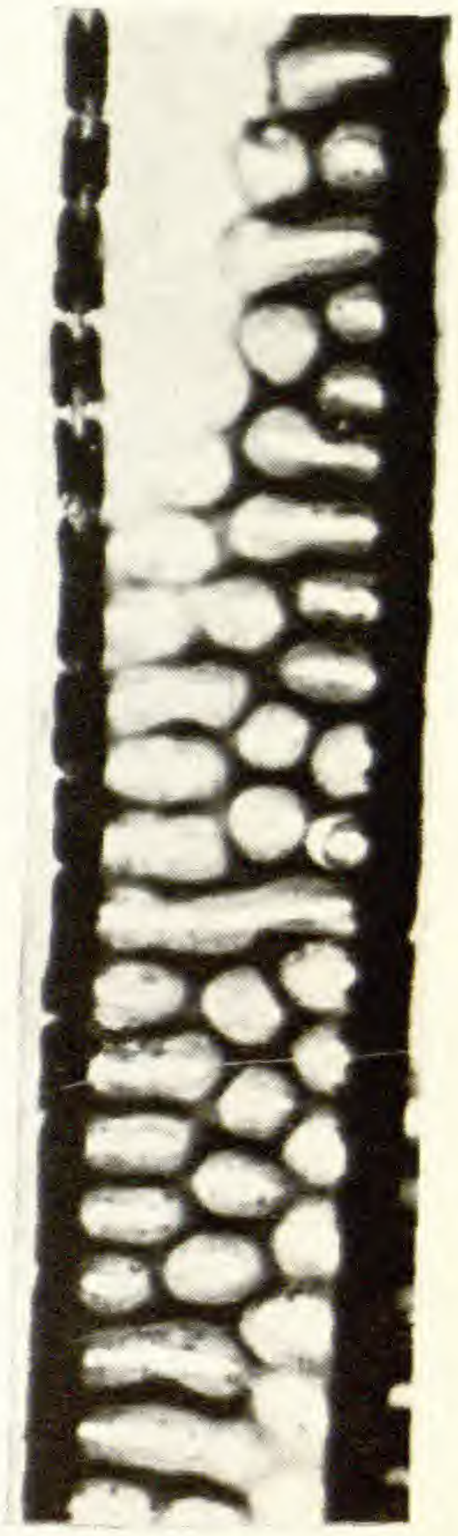

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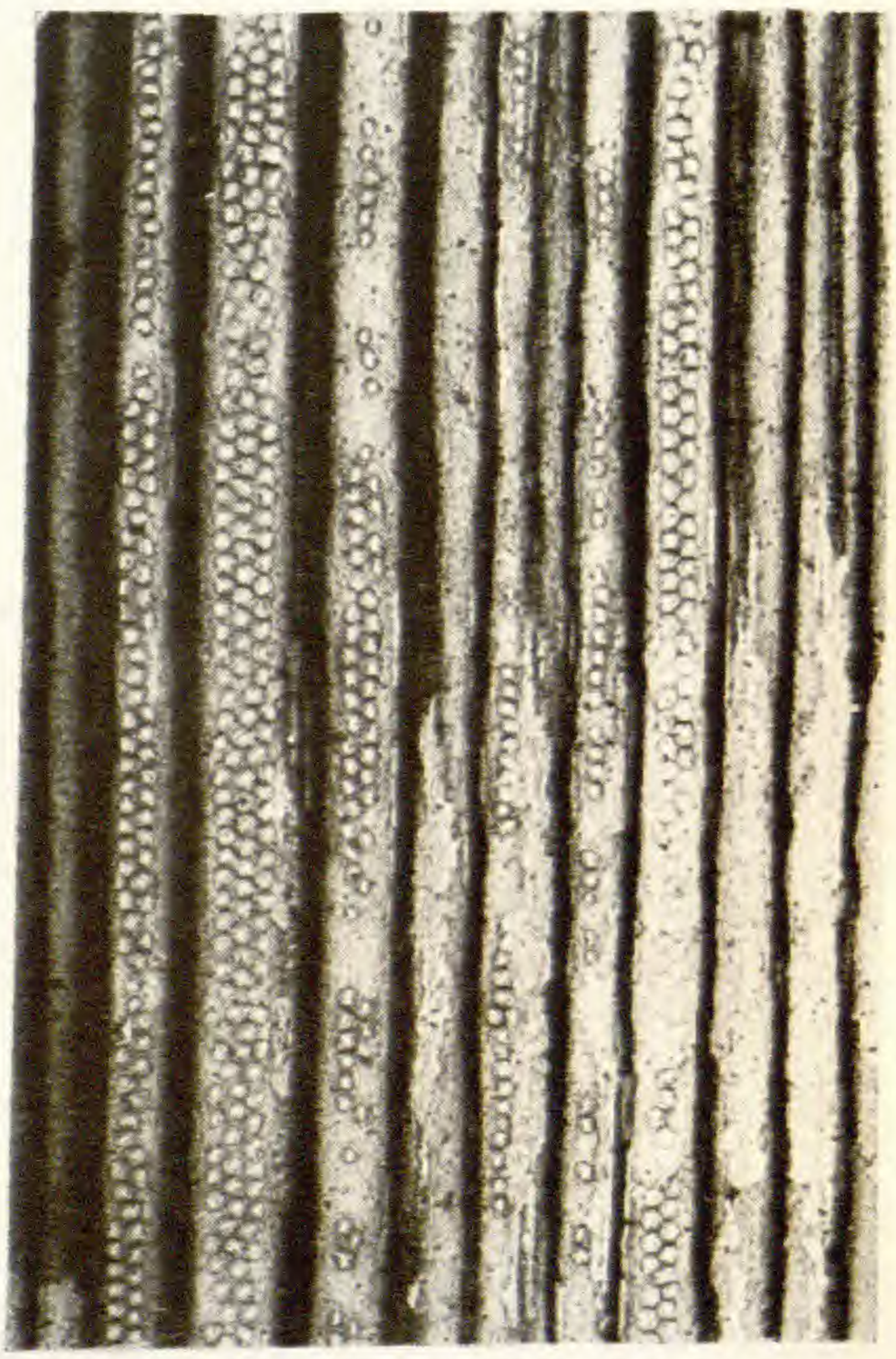

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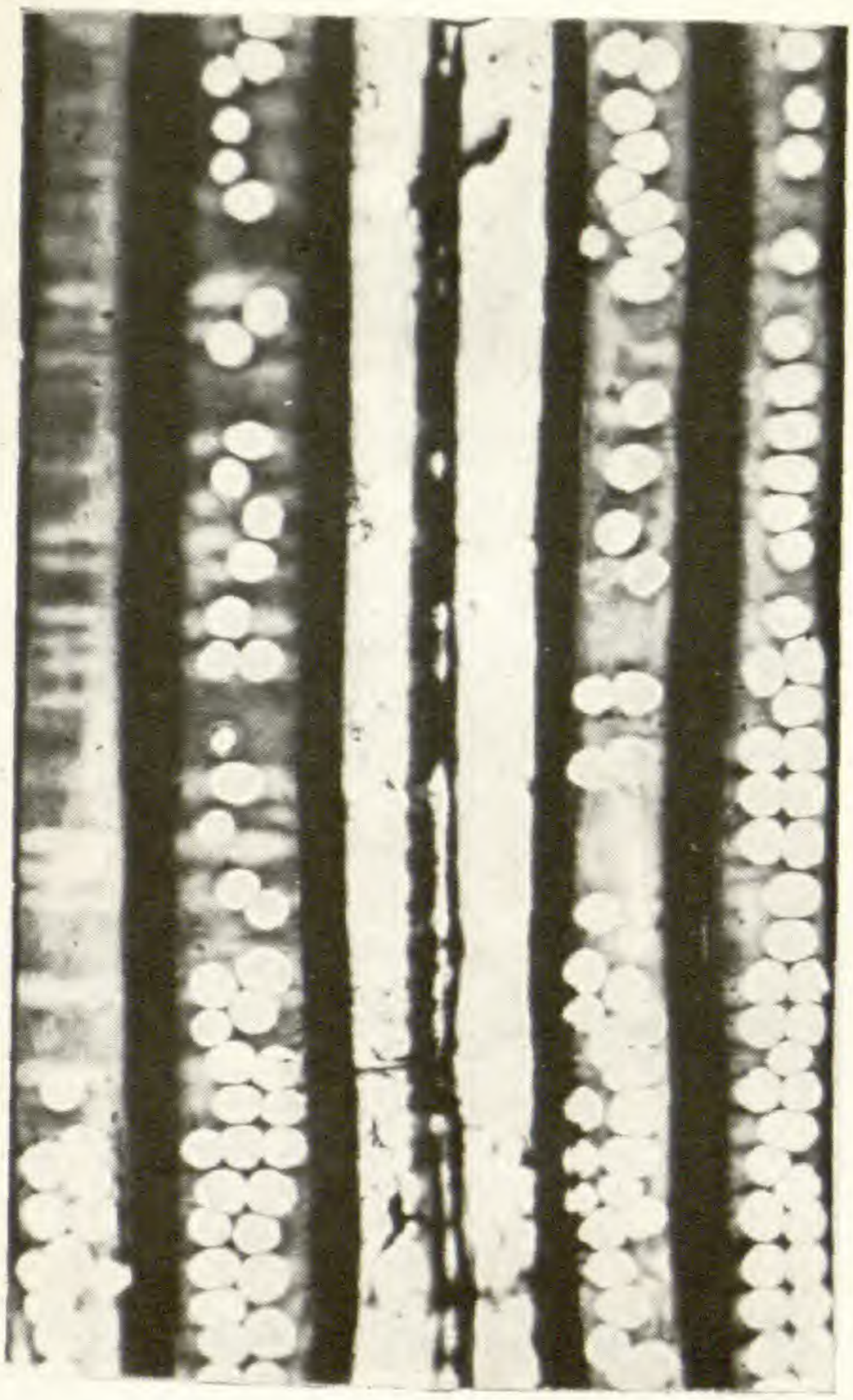

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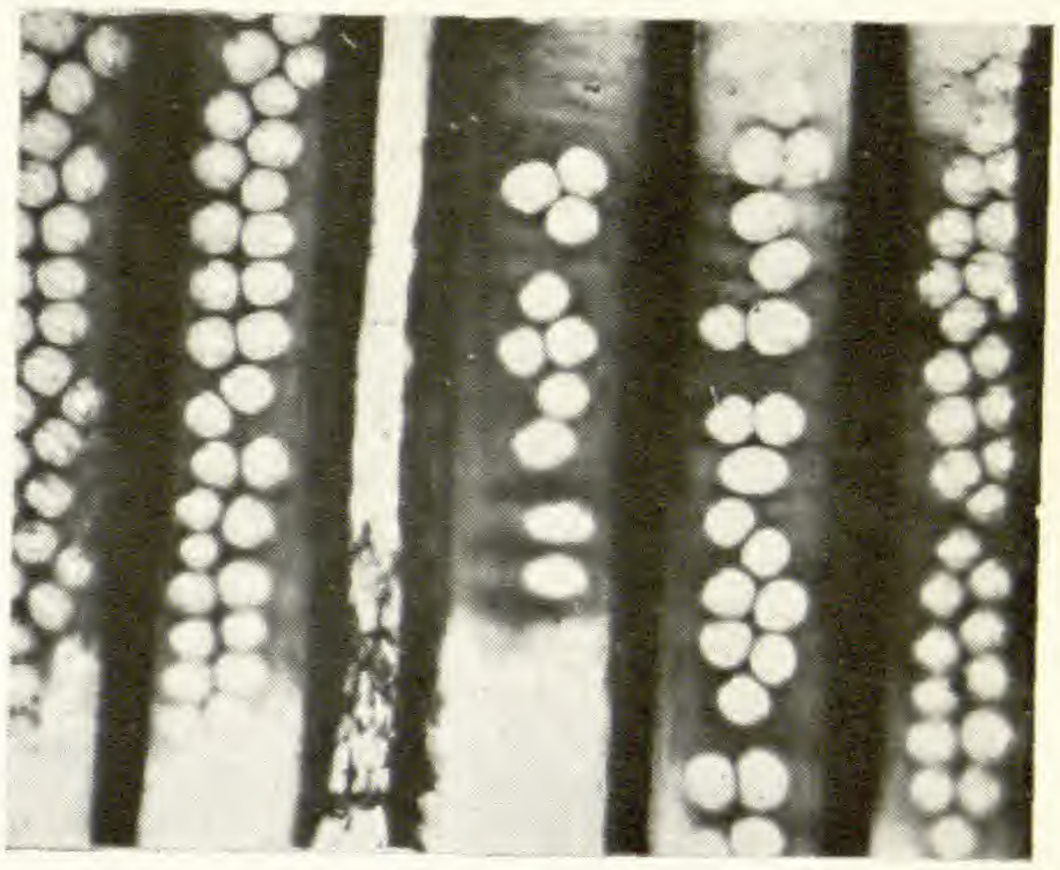

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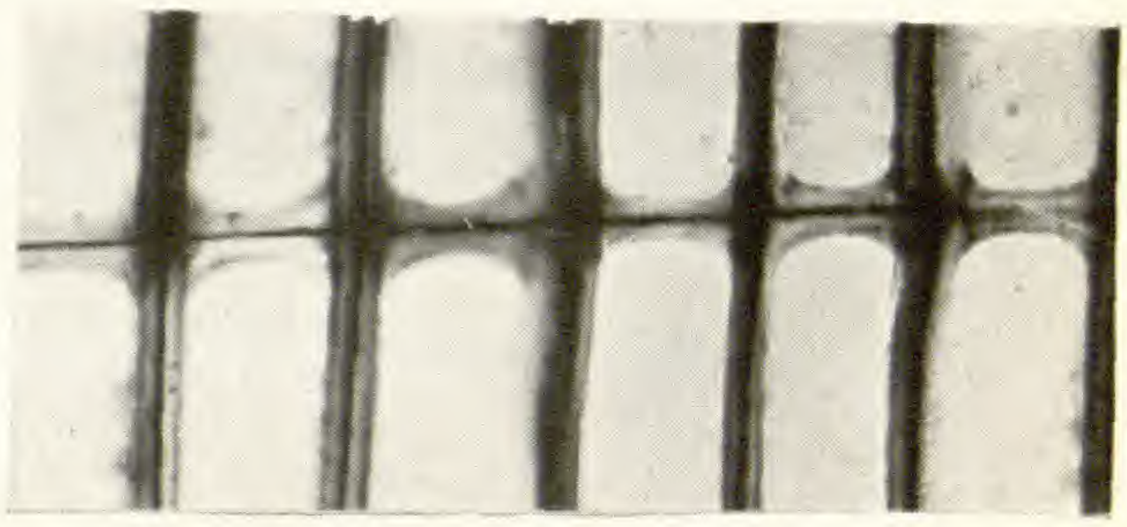

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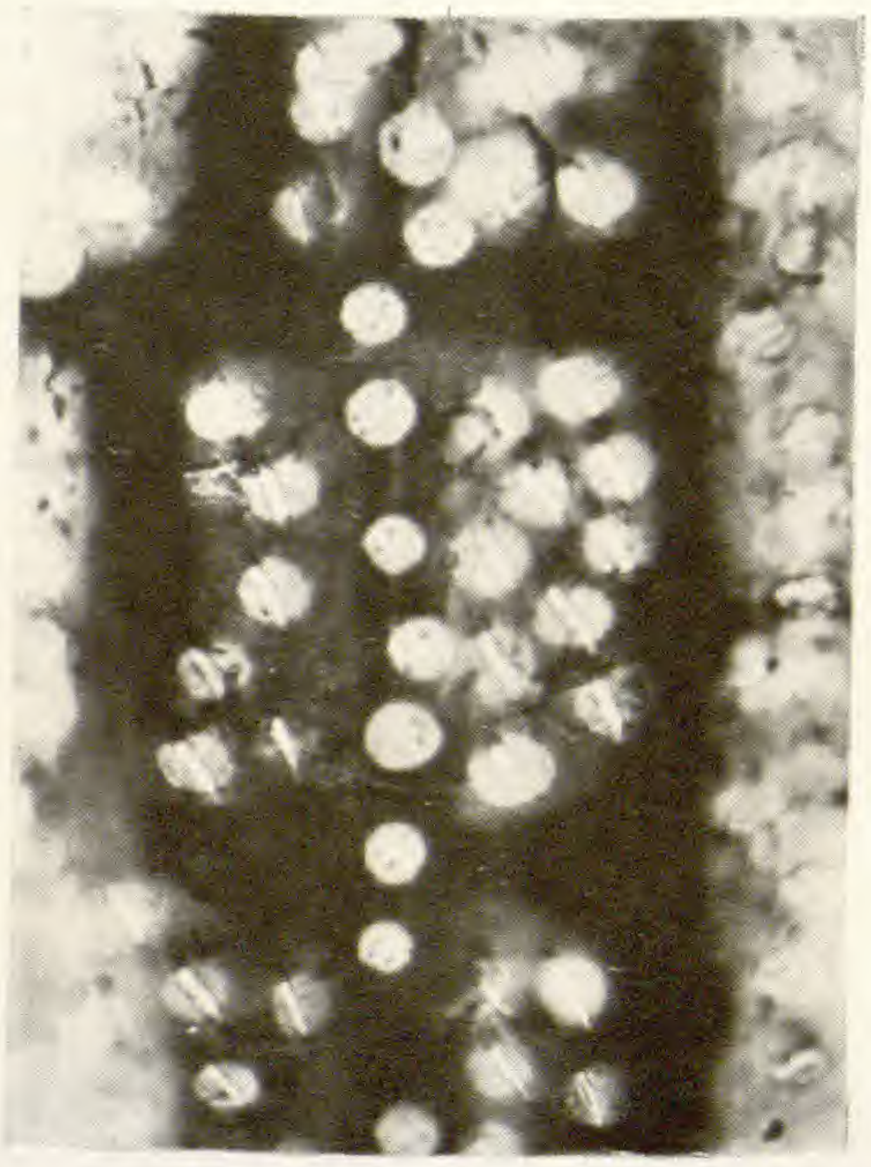

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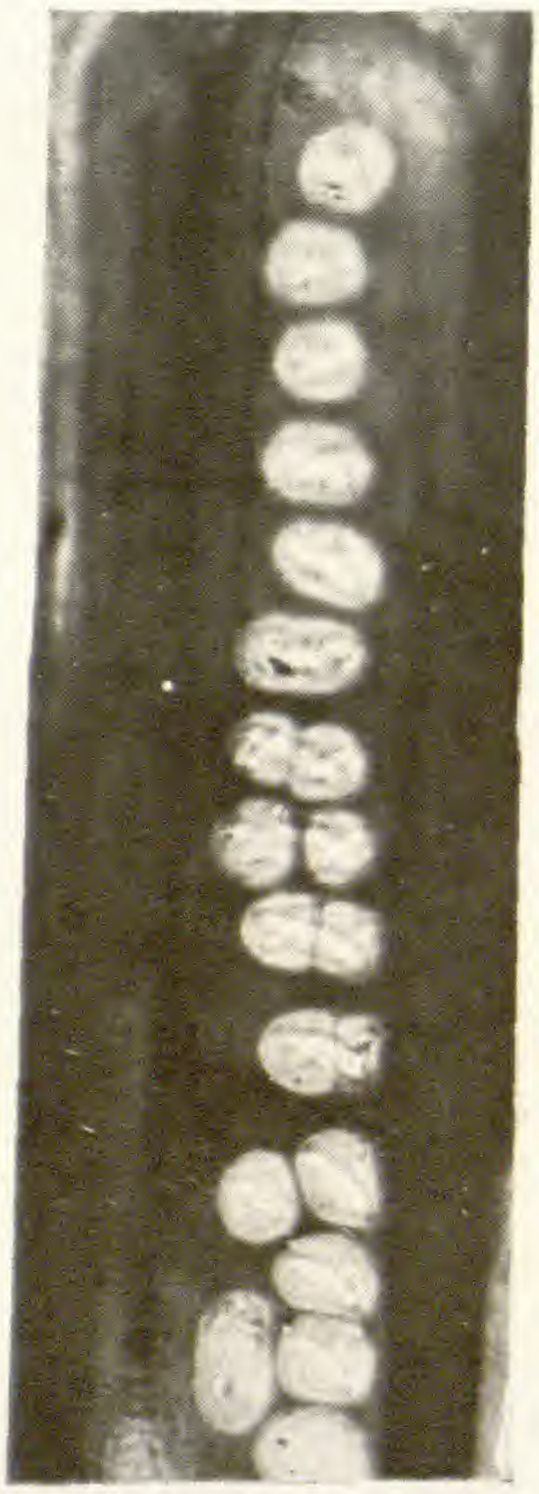

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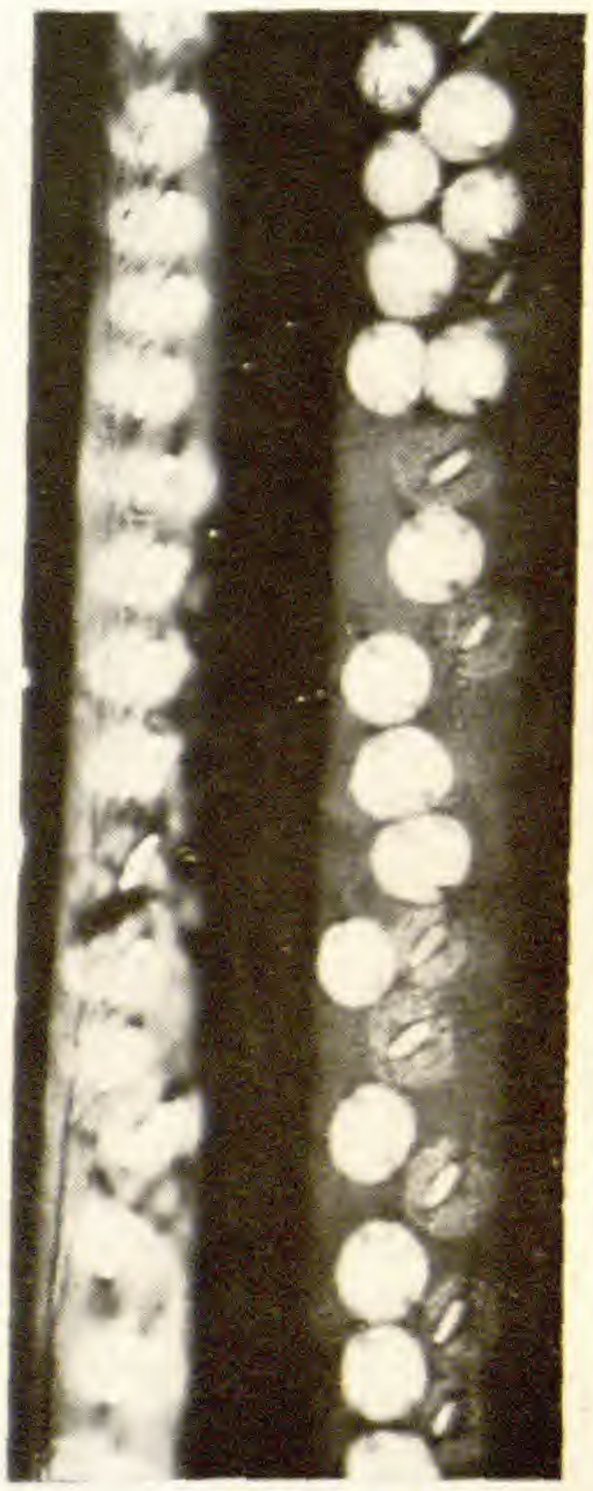

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FIG. 7.—*Dioon spinulosum:* radial section of secondary wood of stem, showing pit arrangement and bars of Sanio; ×225.

FIG. 8.—*Dioon spinulosum:* radial section of secondary wood of stem, showing trabeculae; ×225.

FIG. 9.—*Dioon spinulosum:* radial section of secondary wood of stem, showing pitting in contact with medullary ray; ×445.

FIG. 10.—*Dioon spinulosum:* radial section of secondary wood of stem, with fusion pits near end of tracheid; ×445.

FIG. 11.—*Dioon spinulosum:* radial section of secondary wood of stem, showing pitting in contact with wood parenchyma, and unilateral pits between two tracheids; ×445.

DEVELOPMENT OF EMBRYO OF GNETUM

HULDA I. HAINING

(WITH PLATES XXXIX–XLI AND ONE FIGURE)

In his paper on the morphology of *Gnetum*, THOMPSON (**6**) fully described the different phases in the reproduction of this genus, except the development of the embryo. The material for an investigation of the latter subject was turned over to me, and the results of my study are given in the following pages.

The species studied included *G. funiculare*, and *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 of the Buitenzorg Botanic Garden. Certain stages were also studied in *G. Gnemon*. The young stages and thicker parts of the embryo were cut in serial section. Preparations of the ripe red fruit and germinating seeds of *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 were made by dissecting out the embryos with their tortuous suspensors, which were then stained in Delafield's haematoxylin, extended, and mounted in balsam. On account of the widely branching character of the suspensors this method could not be used for *G. funiculare* and *G. Gnemon*, and it was necessary to make serial sections.

History

BOWER (1) gives the following account of the history of the work on the embryo of *Gnetum*, prior to his own study of the subject. In 1832 BLUME and GRIFFITH had observed in *G. scandens* and *G. latifolium* respectively that within a cavity in the endosperm a coiled mass of suspensors is formed bearing an embryo with two small cotyledons. HOOKER examined sections of *G. Gnemon* and found therein "tubular cells" which occasionally branch, permeating the apical part of the endosperm.

In 1882 BOWER published his account of the development of the embryo of *G. Gnemon*. He states that in the ripe seeds the suspensors bear no embryo, but have the appearance described by HOOKER. Although doubtful of the origin of the first cell of the embryo, formed after germination, he assumes that it is cut

off from the apex of the suspensor. Then he describes the formation of the multicellular embryo, in which an apical cell functions. No mention is made of long secondary suspensors, such as those observed in *G.* sp. 15 and *G.* sp. 59; but the description of the differentiation of the tissues in the embryonic body is in accord with that given later for these species. Reference is also made to seeds of an unknown species in which the suspensors form a coiled bundle in a cavity in the endosperm. This corresponds to the condition found in the ripe fruit of *G.* sp. 29; but it is stated that in the individual suspensors a single large nucleus appears toward the tip of the tube, and no reference is made to the peculiar cell which is present in *G.* sp. 29.

LOTSY examined *G. Gnemon* in 1899. He described the extensive suspensor-like elongation of the fertilized egg, the branching of this tube, and the cutting off of an embryo cell at the tip.

COULTER (3) reinvestigated the early stages in the development of the same species in 1908. He found that in the formation of the suspensor free nuclear division takes place, resulting in a few nuclei scattered along the suspensor, which are often separated by transverse walls. He describes a terminal embryo cell containing one of the free nuclei which continues to divide, accompanied by cleavage walls until a multicellular embryo is formed. This account differs from that given by BOWER in this respect, that the earlier writer described an apical cell as functioning, and no free nuclear division.

In 1916 THOMPSON (6) published observations on the embryo of several species of *Gnetum*. Differing from LOTSY's account, he states that the fertilized egg of *G. Gnemon* divides into two cells, both of which develop into suspensors without transverse cleavage walls. In *G.* sp. 33 he describes a proembryo consisting of a small group of irregularly arranged cells, produced by division of the fertilized egg. Each of these cells then elongates, forming a tortuous suspensor which contains normally only one nucleus, although the possibility of the occurrence of several nuclei would not be excluded in all cases. Finally, he observed the suspensors of *G. moluccense* growing outside of the endosperm, between it and the nucellus. He states that the tips of the tubes enlarge within the

endosperm, and a group of cells is figured at the end of one of the suspensors,

Description

The early post-fertilization stages have not been satisfactorily determined by the writer, who has not observed the number of divisions of the fusion nucleus. The earliest stage which was recognized is that represented in the reconstruction of *G. funiculare* (fig. 1). This shows a large single-celled proembryo, from several points of which there have grown suspensors. Fig. 3 shows a later stage. While some of these tubes grow directly through the endosperm, others turn over the upper part of the embryo sac and make their way down the opposite side. These suspensors often form a tangle in the narrow part of the embryo sac. THOMPSON has stated that in *G. moluccense* suspensors are found between the endosperm and the nucellus. Several times the writer has observed the same condition in *G. funiculare*. In this species sometimes a tangle of tubes is observed in that position. At certain distances, cross-walls are formed in these suspensors, and branches grow into the endosperm (fig. 17). The ends of the suspensors in the endosperm have the usual dense protoplasm and nucleus.

In the earlier stages of the development of the embryos of *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 the endosperm becomes rather disorganized by the growth of tubes, so that in the ripe seed a central corrosion cavity is found. Packed in this is a coiled bundle of tubular structures (fig. 4). When these tubes are extended they measure about 23 mm. They have thick walls of a gelatinous nature. In *G. Gnemon* and *G. funiculare* no such coiled bundle of suspensors is found; they soon separate from each other and branch widely through the endosperm. Toward the tip of the suspensor in *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 the protoplasm becomes denser. It surrounds a peculiar, elongated, pear-shaped cell with a deeply staining nucleus and granular protoplasm. The apex of the cell is closely applied to the wall of the suspensor (fig. 9). A short distance from this end cell there appears an extremely large nucleus with a dense nucleolus showing one or more dark colored globules (fig. 9*a*). Sometimes two similar but smaller nuclei, closely associated, may be observed before the end cell is differ-

entiated, one of which may function in the formation of that cell. There are no nuclei distributed along the suspensors.

The condition of the tubes just described is that found in the ripe fruit on the trees, and in many of the seeds on the ground. At the next stage the suspensors swell to about five times their original size, and sometimes curve about as in fig. 5. The end cell widens, and moving out from the surrounding protoplasm crowds into a protrusion of the gelatinous wall of the tube (fig. 10). Division takes place first into two cells (fig. 11); then each of these divides again, forming the four cells shown in fig. 12. In this preparation one of the walls is not visible. The cells continue to divide in an irregular manner, forming an ovoid group (fig. 13). Division continues with elongation of the basal cells. The result is the formation of a long ribbon of tissue (fig. 14) which, like the primary suspensors, is folded in the cavity of the endosperm. This secondary suspensor, usually measuring about 13 mm., is formed of rather long thin-walled cells with large nuclei. This multicellular ribbon is very different from the secondary embryonal tubes of Abietineae, and, on the other hand, is not found at all in *G. Gnemon*, according to BOWER and COULTER. At the basal end of this secondary suspensor a chain of smaller cells with denser nuclei is differentiated, which may function in the proliferation of embryos. At the apex of the secondary suspensor the cells are actively meristematic (fig. 15). When the suspensor has lengthened sufficiently, it is the rapid multiplication of this group of cells which forms the massive embryonic body.

This embryonic body differentiates in a manner similar to that described for *G. Gnemon* by BOWER (1). Fig. 22 shows the first stage as it appears externally. At the apex a conical projection is visible, surrounded by a thick ridge, from which the cotyledons develop. Fig. 23 shows the "foot" or "feeder" projecting from the side of the hypocotyledonary stem. In fig. 24 both hypocotyl and "foot" have elongated, the latter having exceeded the former. Growth continues as shown in fig. 25, and the radicle reaches the micropyle. After forcing its way out of the endosperm, the radicle turns downward. Then the hypocotyledonary stem grows rapidly and makes its way out of the endosperm also. Following this, the

cavity is filled in around the "foot," which remains in the seed as an absorbing organ, so that the condition shown in fig. 26 results.

Sections of these embryos show that internally a root tip is differentiated in a position shown by the triangle *r* in fig. 19. A definite epidermis covers the cotyledons, hypocotyledonary stem, and conical apex of the stem. After the procambium bundles have differentiated, the foot or feeder is formed as a lateral protuberance of the hypocotyledonary stem, into which there is a lateral extension of the tissues. The procambium bundles make a deep loop toward the apex. In sections of an embryo at the stage shown in fig. 21, conspicuous rows of cells, forming the laticiferous ducts, are observed. In the region of the root tip these cells are distinguished by their dense contents (fig. 27). Toward the apex they are much longer (fig. 28), but have straight end walls in which thickened portions were not observed. These correspond to the younger ducts in *G. Gnemon* figured by BOWER. Young sclerenchyma cells, with the characteristic paired nuclei, are also present in these sections.

Polyembryony

It has been stated by BOWER that polyembryony is the rule in *Gnetum Gnemon*, as in other members of the group. From the examples of it observed in *G.* sp. 15 and *G.* sp. 59 it is plain that these species are not exceptions. Some of the variations of it are represented by the diagrams in text-fig. 1. In *A* three of the four primary suspensors have developed extensive multicellular secondary suspensors measuring about 13 mm. These three have grown so closely together that at this length one has not established itself as the successful embryo, although the middle one is slightly in the lead. In *B* are shown two competing embryos keeping pace at a length of about 16 mm. The third, having separated from the others, has become aborted at a length of 6 mm., while a secondary suspensor has not developed from the other tube. In *C* only one primary suspensor has developed a secondary one. This one has already become quite massive in structure, indicating that it is to become the mature embryo. A variation of this is shown in *D*. Here two of the embryos have developed secondary suspensors, but one is stunted, so that it is quite evident which is destined to

become the successful embryo. A case of the development of several embryos from one suspensor is represented by *E*. One of the secondary suspensors has developed into a bulky structure with unusually large cells. Toward the apex of this tissue, a number of groups of meristematic cells appear which resemble normal embryos in their development. The formation of these embryos is shown

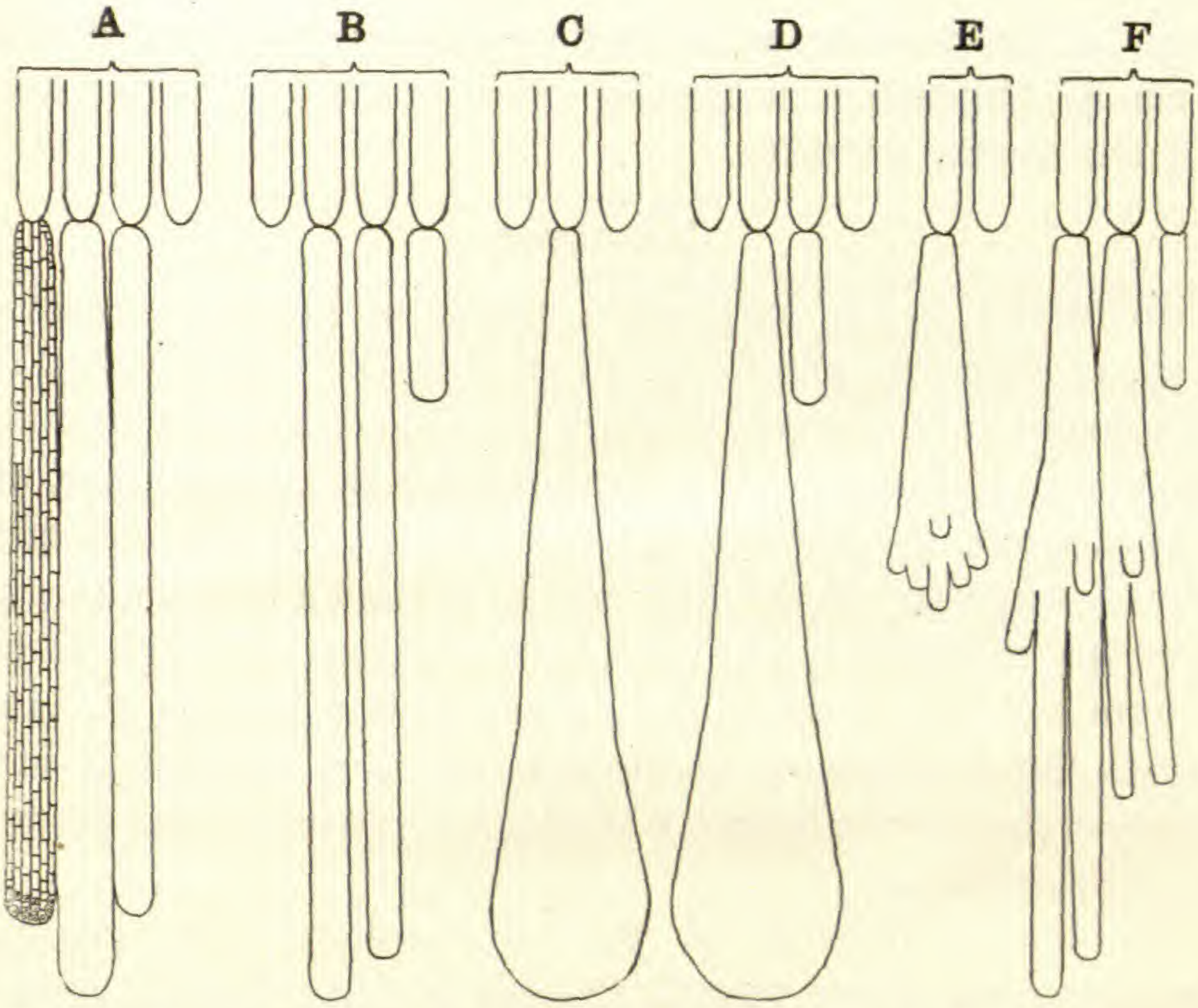

FIG. 1.—Diagrams illustrating polyembryony in *G.* sp. 15 and *G.* sp. 59: upper tubes represent primary suspensors; those below secondary suspensors; *H* and *D* represent usual type; *B* and *C* represent appearance found in small percentage of seeds; *E* and *F* show cases of proliferation of embryos, which are rarely observed.

in fig. 15, which is a small area of fig. 15*a* under greater magnification. The last diagram represents a branching of the secondary suspensor to form extra ones. In the preparation showing this condition the cells are indistinct, but the branches may be considered as prolonged "buds," such as those shown in *E*.

As already mentioned, chains of smaller cells may be observed in the tissues of the secondary suspensors. From these, in a number of cases, outgrowths have been observed which resemble a normal

embryo in development (figs. 6, 7). These cases of budding of embryos represent a process which, according to BUCHHOLZ (**2**), does not occur in the Abietineae. Also, although the secondary suspensors are often closely associated, no case was observed in which they fused to form a single embryo, a process described by BUCHHOLZ as normal in the higher Abietineae, but absent in the pine. From the material examined, it is concluded that in *G.* sp. 15 and *G.* sp. 59, as in other species of the genus as well as in *Ephedra*, (LAND **5**), although a number of embryos may begin to develop, only one reaches maturity.

Discussion

In respect to the long coiled bundle of primary suspensors, *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 are more like the Conifers than such species as *G. Gnemon*, in which the suspensors are widely separated in the endosperm. Furthermore, it is to be noted that, while in *G. Gnemon* the embryo develops directly on the end of the primary suspensor, in the other species studied a long multicellular secondary suspensor is produced, corresponding, although different in form and size, to structures present in the Abieteneae. In all species the development of the external form, as well as of the tissues of the embryo proper, is similar and resembles that described for *Welwitschia* (**4**).

In certain features of the gametophyte and endosperm THOMPSON has shown that *G. Gnemon* is quite different from the other species of the genus, and nearer the angiosperms. It is of course also distinct in its arboreal habit, for the others are all vines. The present study shows that it is different from several other species in the development of its embryo. No long secondary suspensor is formed, and the primary suspensors ramify widely through the endosperm. The reduction in the suspensors appears to bring it nearer the angiosperms. The great reduction in the amount of free nuclear division is a character which separates *Gnetum* widely from the lower gymnosperms. COULTER and BOWER differ as to whether there is any free nuclear division in the formation of the embryo proper at the end of the primary suspensor.

There is certainly none in this position in the other species studied by the writer.

BUCHHOLZ concludes that cleavage polyembryony is a primitive condition in the Abietineae. It is therefore interesting to note that certain species of *Gnetum* retain this condition. There is no fusion of suspensors resulting in a reduction of the number of embryos, such as BUCHHOLZ finds in the higher Abietineae and regards as an evolutionary development. The occasional formation of supernumerary embryos by splitting is evidently of no fundamental significance.

Summary

1. The proembryo of *G. funiculare* consists of a single cell from which suspensors emerge in different directions.

2. Cross-walls and nuclei are formed in these tubes, associated with the branches.

3. In *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 the suspensors form a coiled rope in a cavity in the endosperm; while in *G. funiculare*, as in *G. Gnemon*, they branch widely through the endosperm.

4. In the ripe seeds a peculiar cell is present at the end of the primary suspensor.

5. When germination begins in *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59, a very long multicellular secondary suspensor is formed by division of this cell at the tip of the tube. In *G. Gnemon* no such body appears.

6. The development of cotyledons, root tip, and "foot" at the end of the secondary suspensor is described.

7. Polyembryony is the rule. Several of the primary suspensors usually form secondary suspensors. In some cases these are closely associated and develop equally for some time. In other cases one of the suspensors wins out at an early stage, and the others separate and become stunted. Occasionally the tip of the secondary suspensor divides into a number of branches. Branches from the side of a secondary suspensor are sometimes observed.

8. Except in the reduction of the amount of free nuclear division in all species and in the suspensors in *G. Gnemon* the development is gymnospermic.

To Dr. W. P. THOMPSON I am greatly indebted for his constant interest and advice, and also for all material used in this investigation.

UNIVERSITY OF SASKATCHEWAN
CANADA

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EXPLANATION OF PLATES XXXIX–XLI

PLATE XXXIX

FIG. 1.—*G. funiculare:* proembryo, showing suspensors emerging; *S*, suspensors; *pt*, pollen tube; semi-diagrammatic reconstruction; ×50.

FIG. 2.—*G. funiculare:* proembryo, showing variation of fig. 1; semi-diagrammatic reconstruction; ×50.

FIG. 3.—*G. funiculare:* branching of tubes; *oe*, outside of endosperm; connections in broken lines not determined with certainty; semi-diagrammatic reconstruction.

FIG. 4.—*G.* sp. 29: part of bundle of tubes measuring 23 mm.; ×25.

FIG. 5.—*G.* sp. 15: swollen tips of tubes turning about; ×25.

FIG. 5*a*.—*G.* sp. 15: group of nuclei from tube marked *X*; ×475.

FIG. 6.—*G.* sp. 59: embryo forming from chain of cells in secondary cells (*S*) of secondary suspensor; ×215.

FIG. 7.—*G.* sp. 59: branch of secondary suspensor; ×107.5.

PLATE XL

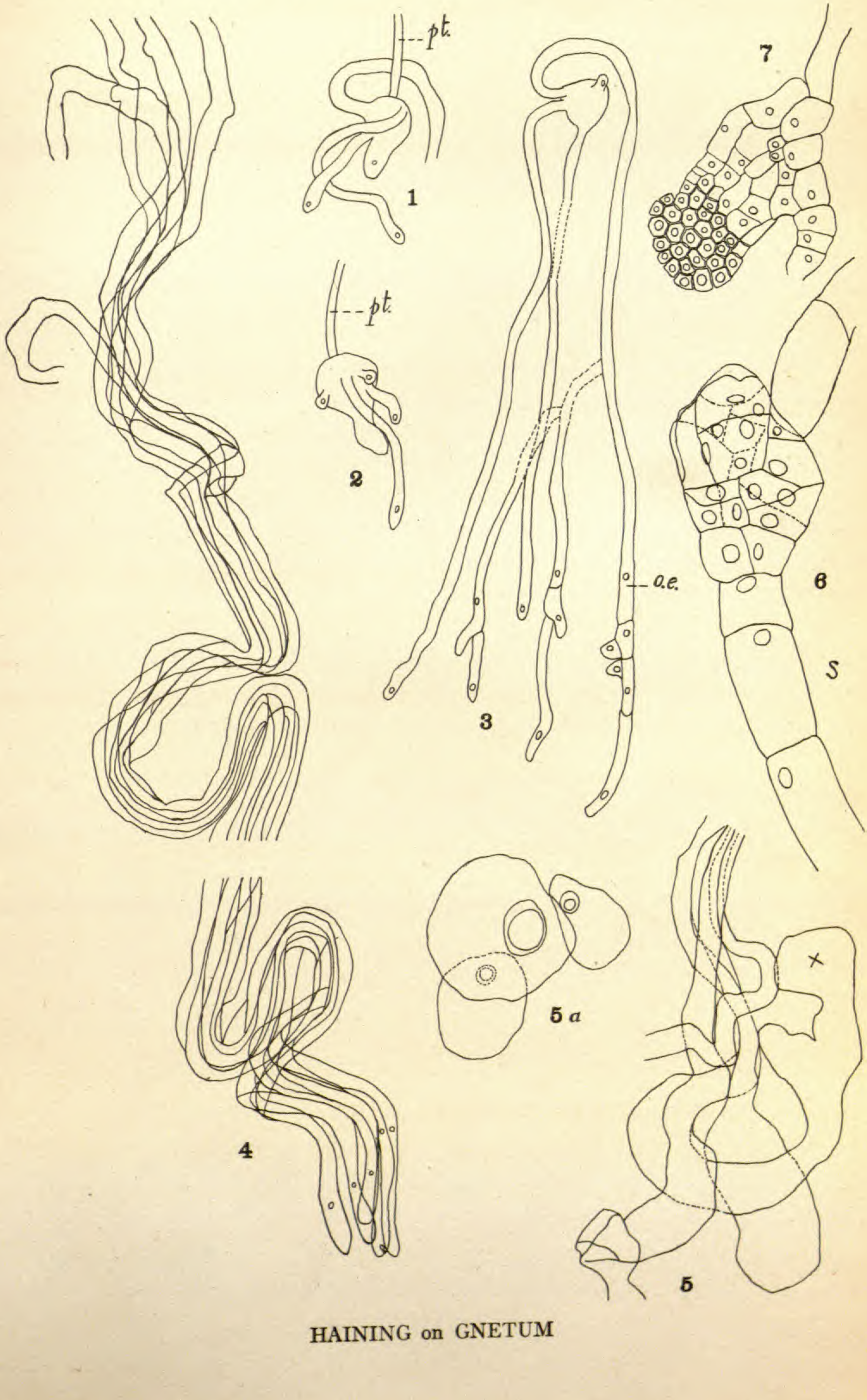
FIG. 8.—*G. funiculare:* branch of primary suspensor in endosperm; ×50.

FIG. 9.—*G.* sp. 29: tip of primary suspensor, showing peculiar cell; ×425.

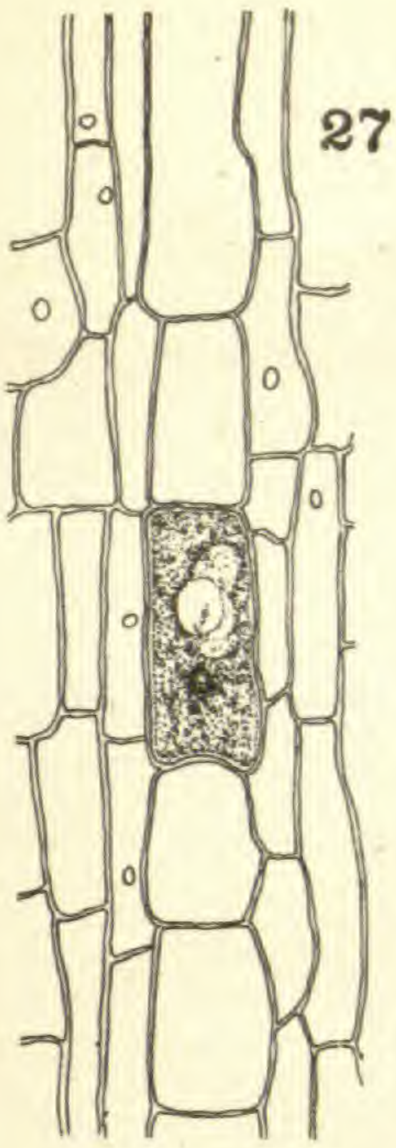
FIG. 9*a*.—*G.* sp. 29: large nucleus of suspensor shown in fig. 9; ×425.

FIG. 10.—*G.* sp. 59: shortened end cell moving out of surrounding protoplasm; *p*, shrunken protoplasm of tube; *w*, wall; ×425.

FIG. 10*a*.—*G.* sp. 59: same under lower magnification; ×50.



HAINING on GNETUM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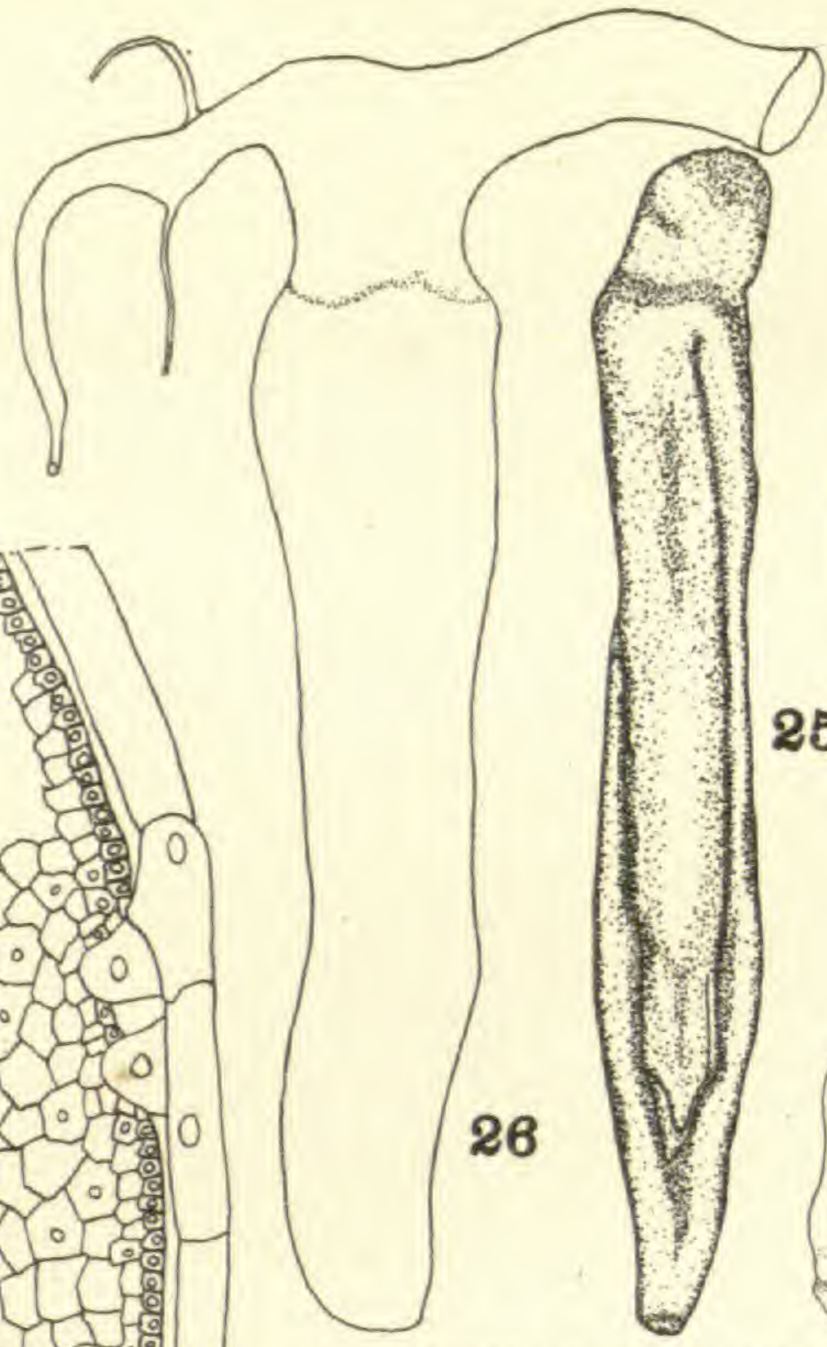
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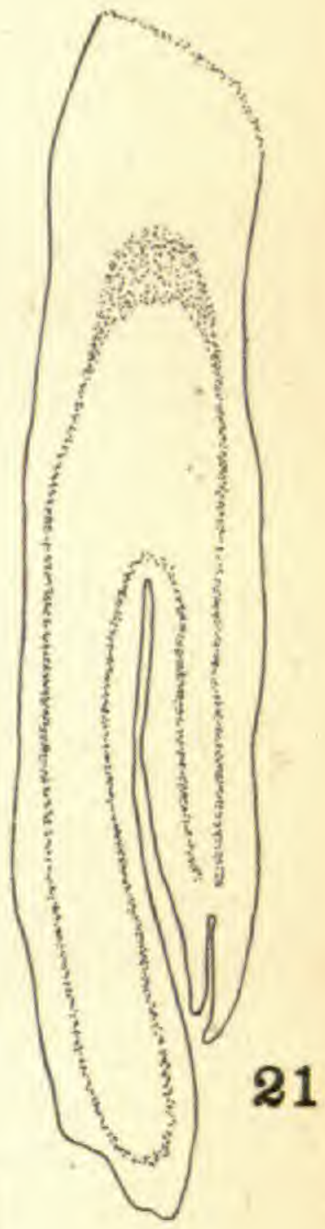
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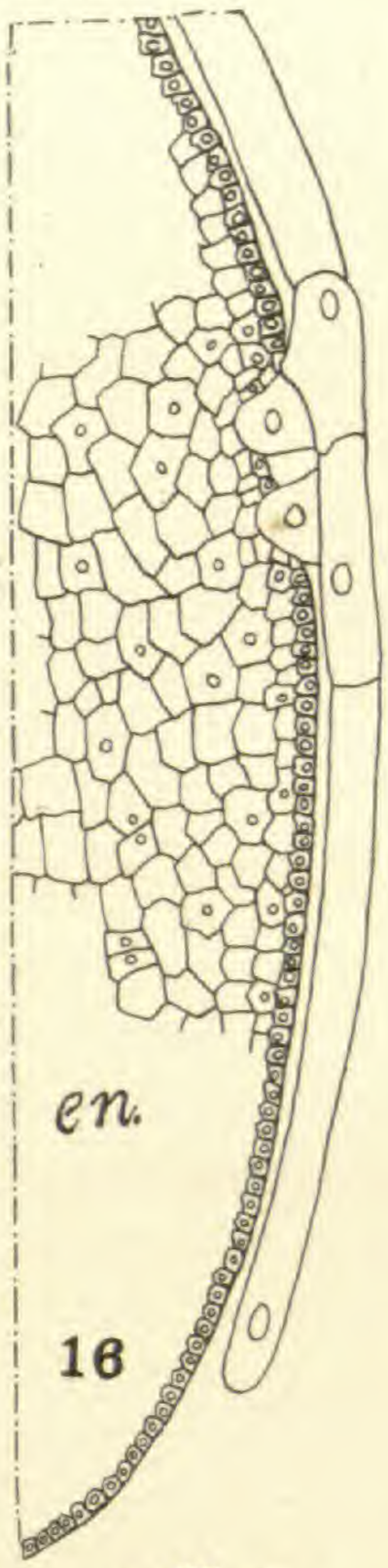
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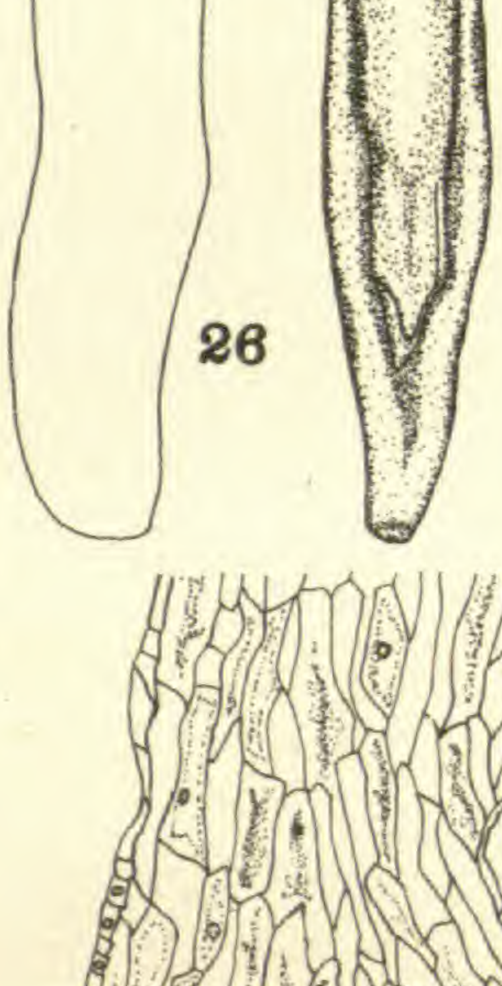
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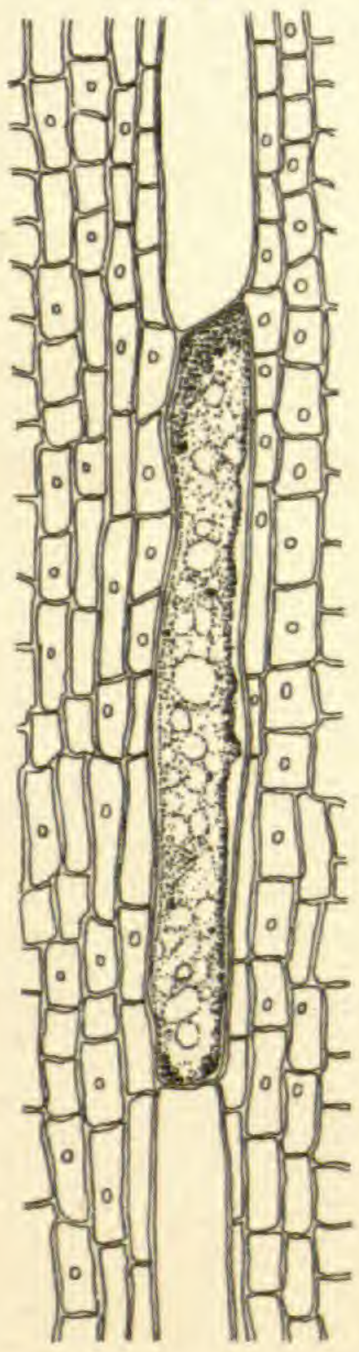
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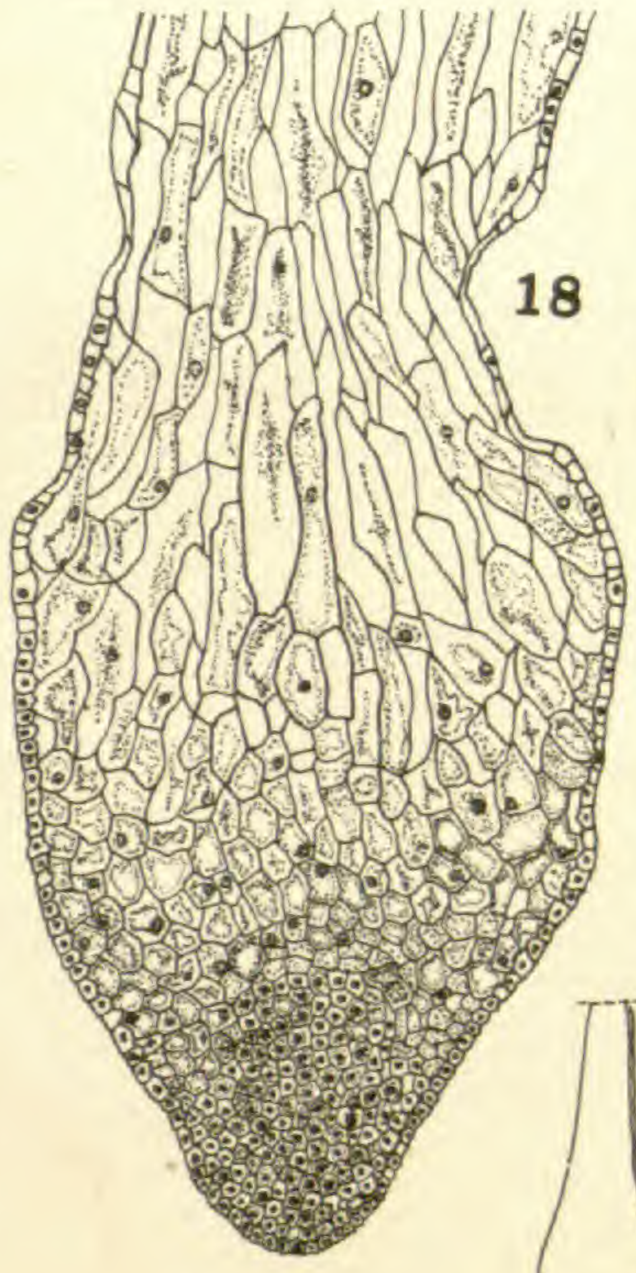
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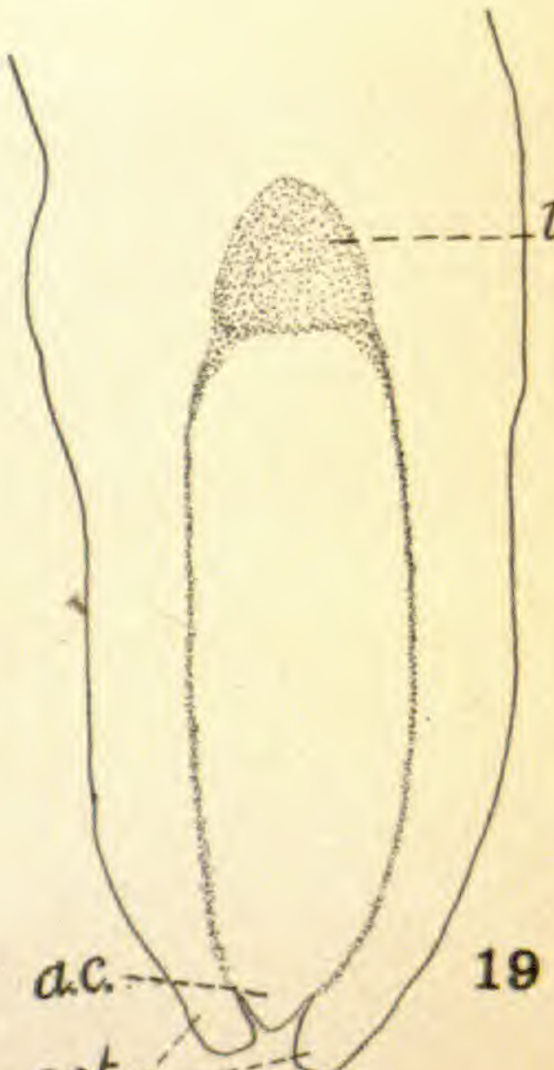
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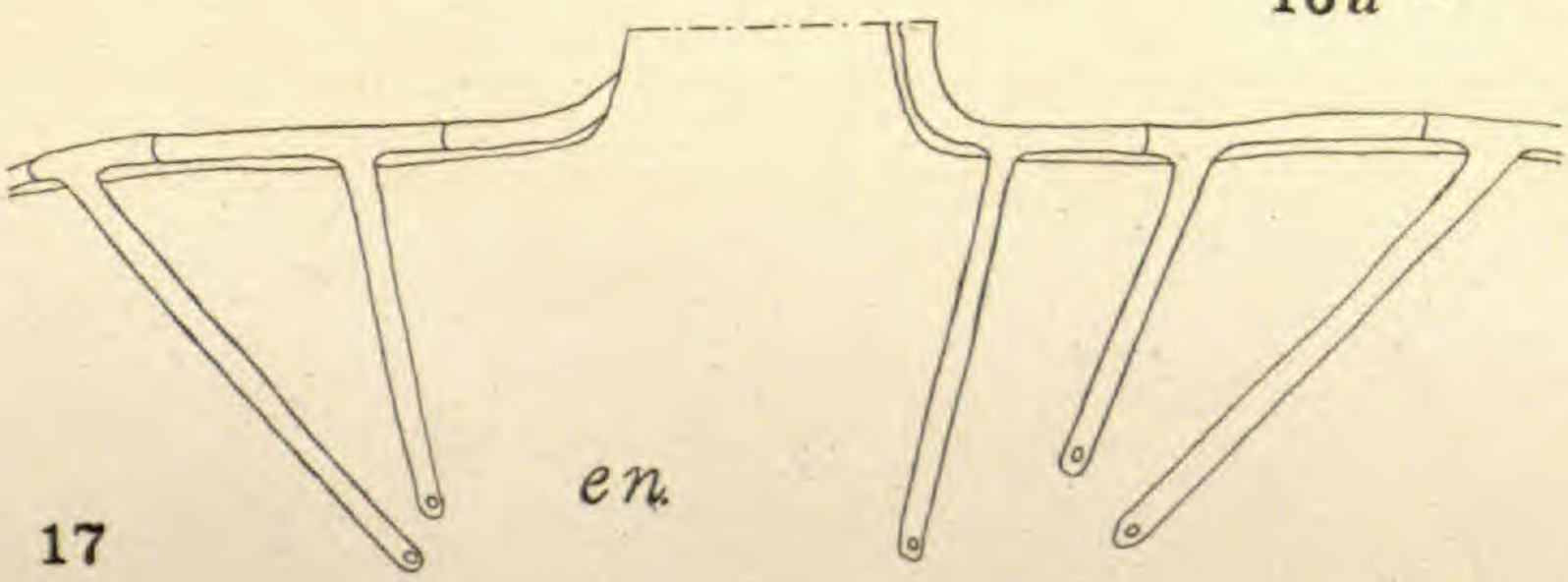
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HAINING on GNETUM

FIG. 11.—*G.* sp. 15: 2-cell stage of secondary suspensor; tube placed to underlie these cells; ×425.

FIG. 11*a*.—*G.* sp. 15: same tube under lower magnification; ×50.

FIG. 12.—*G.* sp. 15: 4-cell stage; ×425.

FIG. 12*a*.—*G.* sp. 15: same tube under lower magnification; ×50.

FIG. 13.—*G.* sp. 59: group of cells irregularly formed; walls too faintly stained to see underlying ones; ×425.

FIG. 14.—*G.* sp. 15: swollen end of primary suspensor, showing ribbon of secondary suspensor; *s*, secondary suspensor; ×25.

FIG. 15.—*G.* sp. 59: groups of meristematic cells at the apex of secondary suspensor tissue; ×50.

FIG. 15*a*.—*G.* sp. 59: same under lower magnification, showing location of groups; ×25.

PLATE XLI

FIG. 16.—*G. funiculare:* suspensor outside endosperm, in immature seed, budding branches; semi-diagrammatic reconstruction; *en*, endosperm.

FIG. 16*a*.—*G. funiculare:* embryo sac, showing position of tube in fig. 16; semi-diagrammatic; ×5.

FIG. 17.—*G. funiculare:* branches penetrating endosperm from tangle of tubes without; in ripe fruit; diagram *en*, endosperm.

FIGS. 18–28.—*G.* sp. 15.

FIG. 18.—Section of tip of secondary suspensor, showing group of meristematic cells and structure of tissue; ×2.5.

FIG. 19.—Section of body of embryo showing stage before "foot" is differentiated; *cot*, cotyledon; *ac*, central apical cone; *r*, root tip; ×25.

FIG. 20.—Section of body of embryo; ×25.

FIG. 21.—Section of older embryo; ×7.5.

FIG. 22.—Embryonic body: gross material; ×7.

FIG. 23.—Embryonic body: cotyledons and "foot" (*f*) differentiated; gross material; ×7.

FIG. 24.—Same, later stage; ×7.

FIG. 25.—Same, later stage; ×7.

FIG. 26.—Root and hypocotyledonary stem outside of seed; ×7.

FIG. 27.—Longitudinal section of young laticiferous cells in basal part of embryo; ×107.5.

FIG. 28.—Longitudinal section of laticiferous cells from nearer apex; ×107.5.

A MORPHOLOGICAL STUDY OF CICER ARIETINUM

THEO. HOLM

(WITH PLATES XLII–XLIV)

Cicer arietinum has an interesting history; the generic name was proposed by PLINY, the specific by DODONAEUS, and both were accepted by LINNAEUS. Of the seven known species of the genus, *C. arietinum* is the only one of economic importance. It has been cultivated for many years, and at present it is not known in its wild state. According to DE CANDOLLE[1] it was evidently introduced from the Orient, and this writer has given an interesting account of its history. At the time of HOMER the plant was cultivated by the Greeks under the name "erebinthos"; by DIOSCORIDES it was called "krios," on account of the seed resembling somewhat the head of a ram; the Romans called it "cicer," from which the names "chiche" (Italy) and "pois chiche" (France) are derived; the name "kikere" is used by the Albanians. Although extensively cultivated in Egypt since the beginning of the Christian era, it does not seem to have been known to the ancient Egyptians. The meaning of the names used in Spain ("garbanzo" or "garbantzua") is uncertain, being neither Arabian nor Latin. In Sanscrit it is called "chennuka," and according to BRETSCHNEIDER the plant was cultivated in China in the fourteenth century under names indicating its introduction from the west. In Greece the dried seeds are salted and roasted, and are known as "stragalia" according to HELDREICH.[2] In our western states the seeds are used for coffee, hence the name "coffee-pea." For several years I have grown the plant in my garden at Brookland, D.C. Although it is a member of the Vicieae, it shows a very peculiar habit, and the structure is interesting in several

[1] DE CANDOLLE, ALPHONSE, Origine des plantes cultivées. Paris. 1886.

[2] HELDREICH, TH., Die Nutzpflanzen Griechenlands. Athens. 1862.

respects. No mention of this plant is made by SOLEREDER in his comprehensive work *Systematische Anatomie der Dicotyledonen*.

C. arietinum is an annual, branching from the base, the several stems erect, angular or winged, densely glandular-pubescent, as are also the leaves; the primary root is quite strong, and the lateral roots bear tubercles. The leaves are odd-pinnate with obovate, dentate leaflets (fig. 4), and the stipules are incised (fig. 6). The solitary flowers (figs. 4, 5) are white, with the wings free, and they are borne on axillary peduncles, strongly reflexed; the two prophylla are very distinct. The sessile ovary contains one or two ovules, and the filiform style is incurved. The pod is relatively large (fig. 6), ovoid to oblong, turgid, 2-valved; the seeds (fig. 7) are subglobose with the radicula almost straight. As stated, the plant is glandular-pubescent, and the hairs are clavate and pluricellular (fig. 8). They contain free oxalic acid, according to VAN TIEGHEM.[3]

Seedling

So far, no description has been given of the seedling stage of this plant. The seeds require only a few days (less than a week) to germinate; a young seedling is shown in fig. 1. The primary root (*R*) is quite long, vertical, and branches freely. There is no hypocotyl, and the cotyledons (*Cot*) remain subterranean, each with an axillary bud. The plumule develops an erect shoot, the first leaves of which are merely stipules. Fig. 2 illustrates an older seedling with the primary root still longer, and with the cotyledonary buds having developed into small shoots (fig. 3). The primary shoot now represents an erect stem with typical foliage, and several axillary branches.

It is characteristic of the seedling stage, therefore, that the cotyledons remain underground, subtending axillary buds; that the epicotyl (*Ep*) is erect and stretched; and that already the first stem leaves subtend branches. The glandular pubescence appears at the seedling stage, but is not shown in the figure, since the hairs cover the stem and leaves completely, and drawn in ink they would make the figures completely black.

[3] VAN TIEGHEM, PH., Traité de Botanique. Paris. 1884 (p. 542).

Internal structure of vegetative organs

ROOT SYSTEM

The roots are neither contractile nor developed as storage roots; they are simply nutritive and, although the plant is an annual, they increase quite considerably in thickness. This increase takes place by means of the activity of the pericambium, developing cork and a secondary cortex, beside by the development of cambial strata inside the stele, in the manner typical of dicotyledons. When fully matured the roots are very strong, owing to the abundance of stereïds outside the primary as well as outside the secondary leptome, and in the secondary hadrome. The primary structure may be studied from the apical portion of the primary root of the seedling. The epidermis is hairy, but there is no exodermis, and the cortex represents a compact parenchyma of about ten layers without deposits of starch. The endodermis is thin-walled, with Casparyan spots plainly visible. The pericambium consists of a single continuous stratum, but is separated from the stele proper by a layer of thin-walled parenchyma. Inside this parenchyma are four strands of stereome with leptome on the sides and on the inner face, beside four rays of hadrome, a small pith occupying the center of the stele. With regard to the hadrome, the protohadrome vessels are annular and reticulated, mostly two side by side, and much narrower than the inner, which vary from reticulated to porous. At this stage there are no signs of cell division in the pericambium, but narrow arches of cambial strata appear between the leptome and hadrome, and none outside the protohadrome vessels. The primary root is thus tetrarch, and the increase in thickness commences by the development of cambium between the leptome and hadrome.

Examining this same root in its older portion near the base, the following structure may be seen. Fig. 9 shows a diagram of the stele and part of the peripheral tissues, of which epidermis, cortex, and endodermis exhibit the same structure as described. Concerning the pericambium there is now a slight indication of increase, demonstrated by a tangential division (but only one) in each cell. The stereome is now more thick-walled (fig. 10, *St*), and there are many layers of cambium on the inner face of the leptome, also

outside the protohadrome vessels, giving rise to several strata of thin-walled parenchyma. This parenchyma formed outside the four strands of hadrome becomes the first medullary rays (fig. 11, *M*). This more advanced stage is readily seen in fig. 9, where the cambial strata (*Camb*) are very conspicuous, beside the commencement of the formation of the four medullary rays outside the vessels. The pith remains unchanged, without becoming sclerotic, and contains no deposits of starch. A corresponding structure occurs in the lateral roots, borne on the primary, but the cambium is not so abundant. If we examine the primary root of a mature fruiting specimen, we observe the structure as shown in fig. 11. All the peripheral tissues from epidermis to endodermis inclusive are lost, but replaced by several strata of thin-walled, homogeneous cork (*Co*), and a secondary cortex (*C*) in which the four stereomatic strands are yet distinct. The cork and the secondary cortex are the products of the cell division within the pericambium. Furthermore, the deep medullary rays may be seen, only two of which have been drawn, and these (*M*) proceed from the old hadrome rays (*PH*), where they were formed originally. Secondary medullary rays are also developed. They commence within the secondary hadrome, and are shorter and much narrower than the primary. The stele is now much broader, and contains secondary stereome (fig. 12, *St*) as cells scattered outside the leptome, also among the secondary vessels (fig. 14, *St*). On the other hand, the central portion of the stele, with the four rays of hadrome and the pith, are unchanged, while the primary leptome has become absorbed completely. These secondary formations within the stele, that is, the medullary rays, the stereome, the secondary leptome (*L*), and hadrome (*H*), are all products of the cambium (fig. 9).

Characteristics of the roots of *Cicer*, therefore, are the abundance of stereids; also, that the increase in thickness commences within the stele proper and not in the pericambium. The fact that a cambium becomes developed as a circular band between the secondary leptome and hadrome (fig. 11, *Camb*) shows that further increase in thickness is secured in exactly the same manner as in the collateral mestome strands of a dicotyledonous stem.

STEM

The stems are angular to narrowly winged, densely covered with glandular (fig. 8), long, unicellular, and pointed hairs; the glandular hairs contain free oxalic acid, according to VAN TIEGHEM. The stem structure in general is quite firm, owing to the presence of hypodermal collenchyma and pericyclic stereome. The cuticle is thick, longitudinally wrinkled, and the outer cell wall of epidermis moderately thickened (figs. 16, 17, *Ep*). Corresponding with the angles or wings are hypodermal strands of thick-walled collenchyma (fig. 17, *Coll*), and the cortical parenchyma is compact, about three layers, filled with chlorophyll. There is no endodermis, but a distinct continuous pericycle, which forms arches of stereome, but is only distinct in the older portions of the stem. In young internodes (fig. 15) the pericycle is so thin-walled that it is barely distinguishable. There are about nine primary mestome strands, which are collateral, and separated from each other by meristematic strata in young internodes.

In this meristem secondary formations arise by means of an interfascicular cambium (fig. 16, *Camb*), which begins from the sides of the mestome strands. As shown in fig. 16, a small strand of leptome (*L*) is the first product within this meristem, and later on the cambium continues to develop secondary hadrome, that is, porous tracheids and libriform (fig. 18, *H*). The pith is thin-walled, and not starch bearing, but large spheric crystals were observed, the material having been preserved in alcohol. Similar crystals also occur in the cortex of the old internodes. While the structure of a young internode shows no secondary formations, in the mature stem a compact stele is found in which thick-walled libriform is very conspicuous, developed from the interfascicular cambium.

Finally may be mentioned that the epicotyl of the young seedling is glabrous, with a smooth cuticle, but without collenchyma. The cortex is very thick, about twenty layers, and an endodermis is developed outside twelve arches of pericyclic stereome, corresponding with twelve collateral mestome strands. No secondary formations occur at this stage.

Characteristic of the stem structure of *Cicer*, therefore, is the presence of endodermis in the epicotyl, and its absence from the internodes above; the glandular hairs, very seldom met with in this family; the presence of interfascicular cambium; and the spheric crystals in the cortex and pith.

LEAF

The leaf structure is not exactly bifacial, since the stomata are distributed over both faces, and equally abundant; but the chlorenchyma shows both palisade and pneumatic tissue. Hairs like those of the stem abound on both faces of the blade, and the cuticle is wrinkled above and below the stronger veins; otherwise it is thin and smooth. The epidermis is slightly thick-walled above and below the midrib, and the lateral walls are undulate on the dorsal side (fig. 19), but almost straight on the ventral side. The stomata (fig. 19) have no subsidiary cells; they are free, and raised a little, with a wide shallow air chamber. The chlorenchyma covers both faces of the leaf, and consists of a ventral palisade tissue of three layers (fig. 13, *P*), and of a pneumatic tissue of about five strata (fig. 13, P^+). There is neither collenchyma nor water storage tissue, and the veins are completely imbedded in the chlorenchyma. Around the midrib and the strong secondary veins are parenchyma sheaths, each cell of which contains a large rhombic crystal of calcium oxalate. Moreover, the midrib has a little pericyclic stereome on the leptome side, and consists of a single collateral mestome strand, with leptome, cambium, and a few vessels.

Characteristic of the leaf structure, therefore, is the distribution of the stomata over both faces of the blade; the dense chlorenchyma; the poor development of mechanical tissues; and the crystal bearing parenchyma-sheath. The internal structure of *C. arietinum* thus resembles that of a xerophilous plant among the Papilionaceae, especially when we add the profuse development of hairs, pointed, clavate, and glandular. The species evidently originated in a country with a warm and dry climate, and it has been suggested as possibly between Greece and the Caspian Sea.

CLINTON, MD.

EXPLANATION OF PLATES XLII–XLIV

PLATE XLII

FIG. 1.—Young seedling: *R*, primary root; *Cot*, cotyledons; *Ep*, epicotyl; natural size.

FIG. 2.—Older seedling; natural size.

FIG. 3.—Part of same, showing shoots (*S*) in axils of cotyledons; natural size.

FIG. 4.—Floral shoot; natural size.

FIG. 5.—Flower seen from below; natural size.

FIG. 6.—Pod; natural size.

FIG. 7.—Same in longitudinal section; natural size.

FIG. 8.—Glandular hair; ×480.

PLATE XLIII

FIG. 9.—Cross-section of young root: *Ep*, epidermis; *C*, cortex; *End*, endodermis; *St*, stereome; *L*, leptome; *Camb*, cambium; ×112.

FIG. 10.—Part of same root: *P*, pericambium; other letters as given; ×744.

FIG. 11.—Cross-section of old root: *Co*, cork; *M*, medullary ray; *PH*, protohadrome; *P*, pith; ×90.

FIG. 12.—Part of same root; ×744.

FIG. 13.—Cross-section of leaf: *Ep*, ventral; Ep^+, dorsal epidermis; *P*, palisade tissue; P^+, pneumatic tissue; ×480.

PLATE XLIV

FIG. 14.—Part of root: letters as in fig. 11; *V*, vessels; ×744.

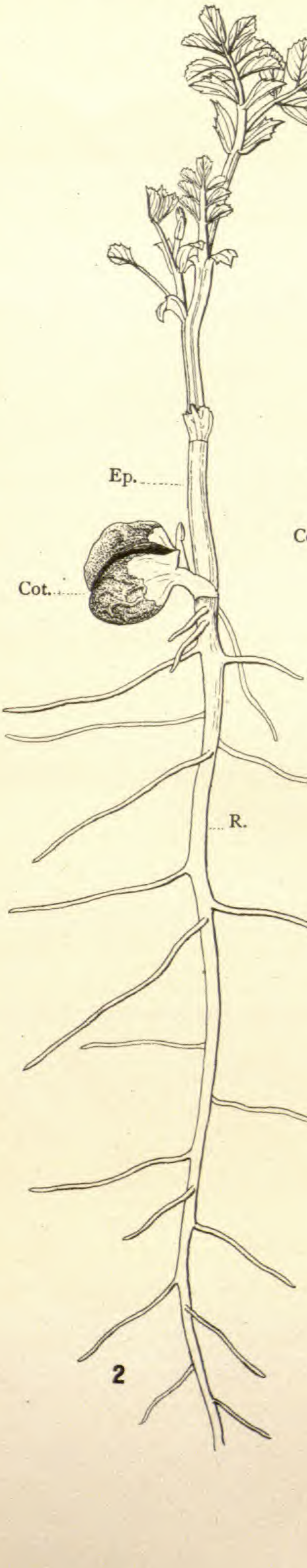
FIG. 15.—Cross-section of stem; ×480.

FIG. 16.—Part of same: *L*, leptome above interfascicular cambium (*Camb*); ×744.

FIG. 17.—Part of same: *Coll*, collenchyma; ×480.

FIG. 18.—Part of same: all tissues of secondary formation; ×480.

FIG. 19.—Stoma of leaf; ×480.



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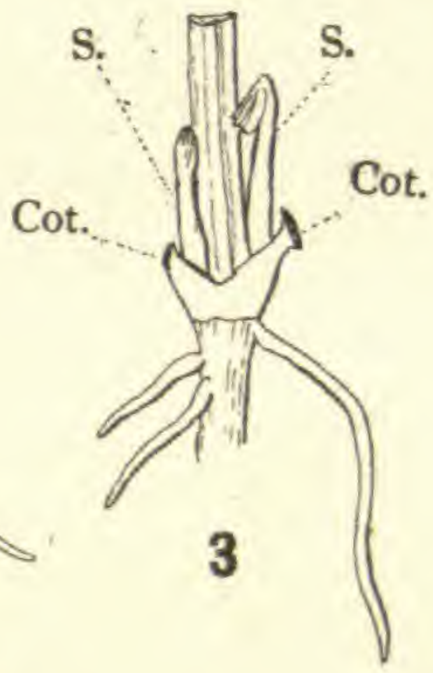

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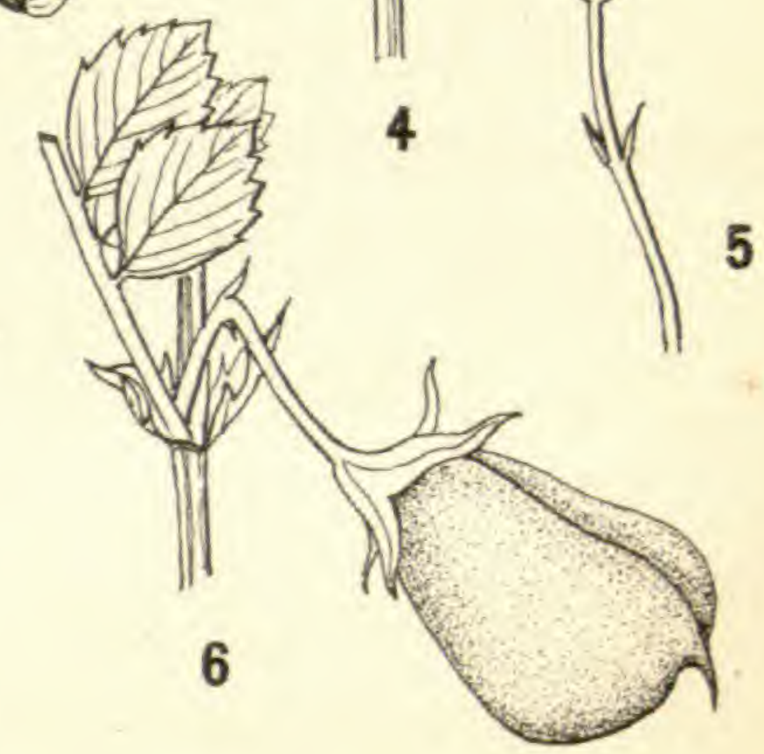

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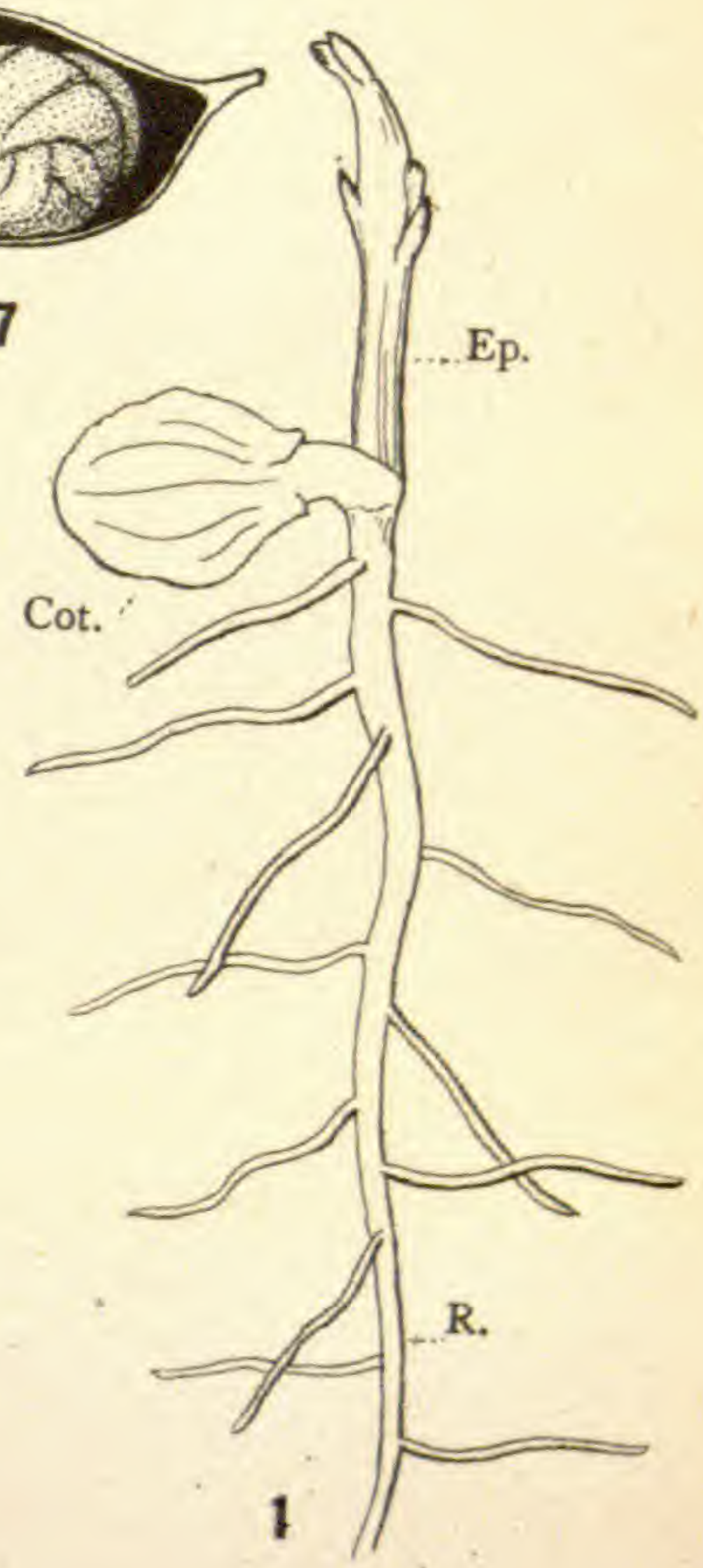

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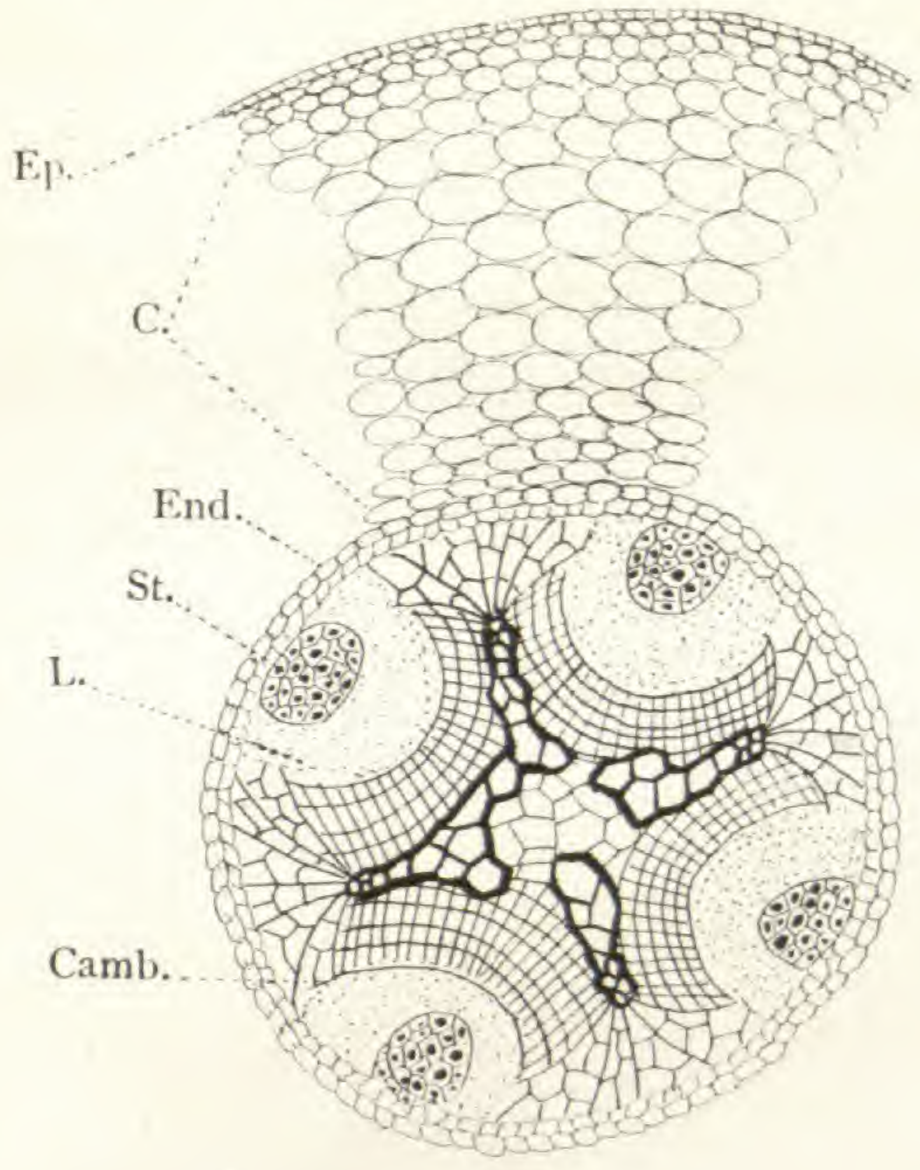

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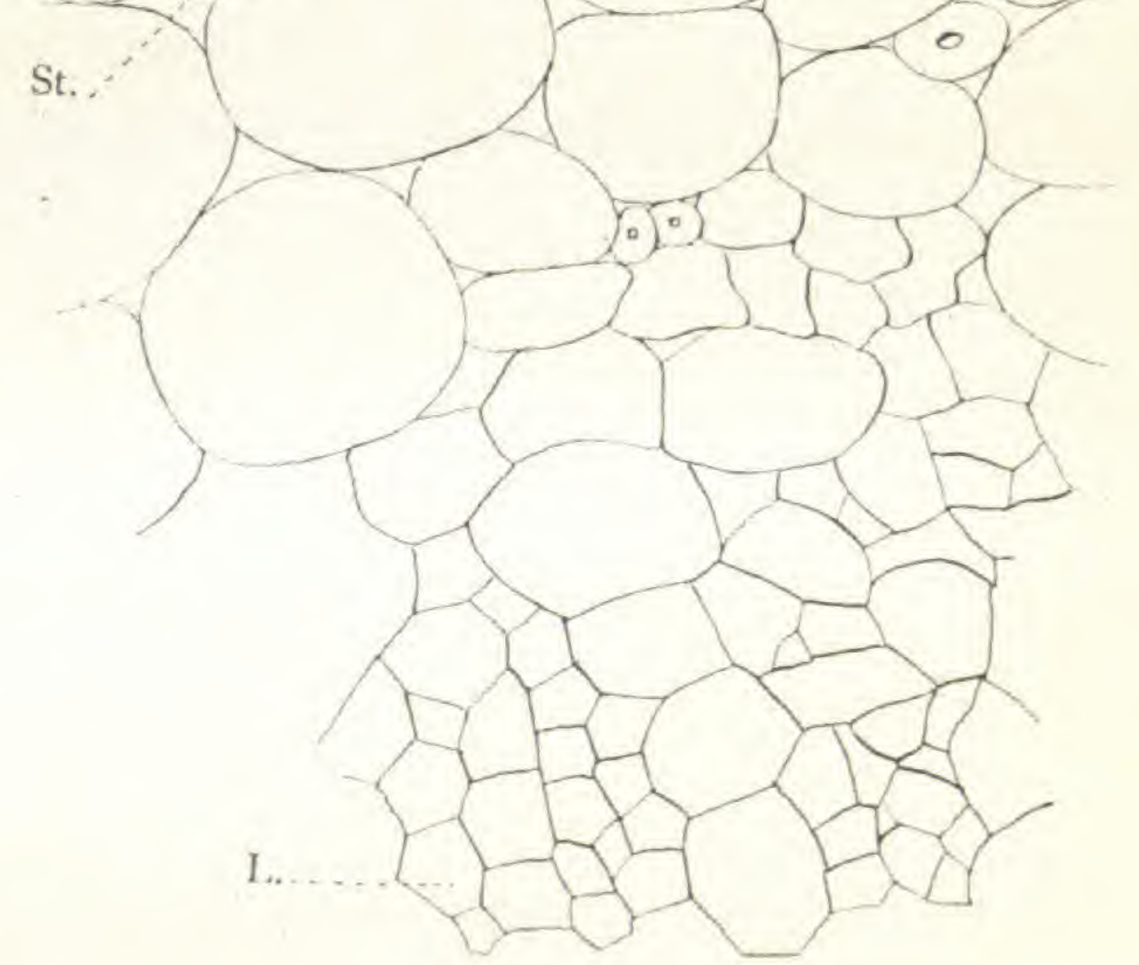

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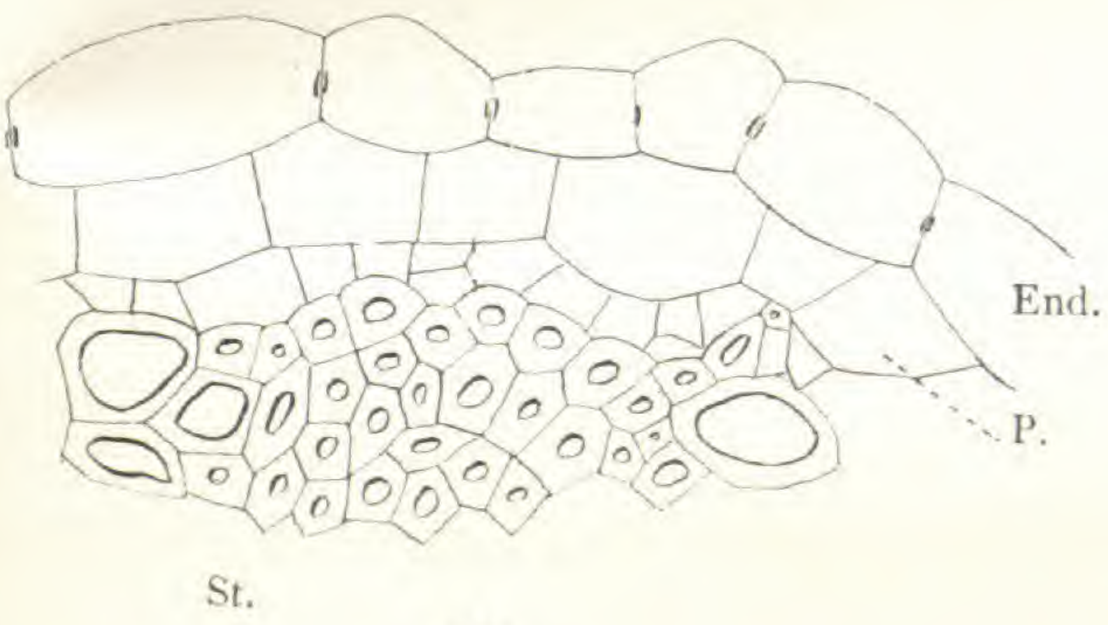

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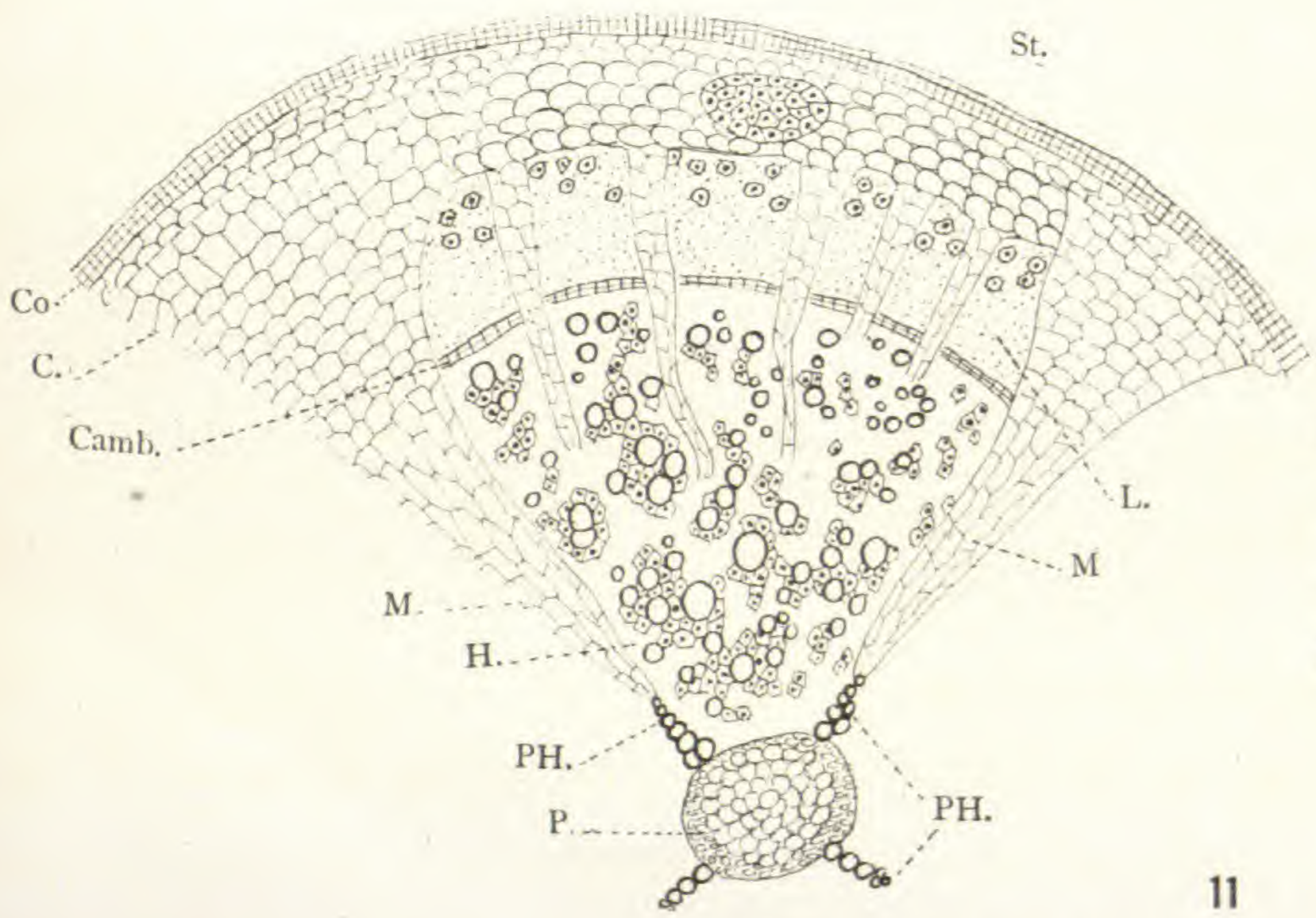

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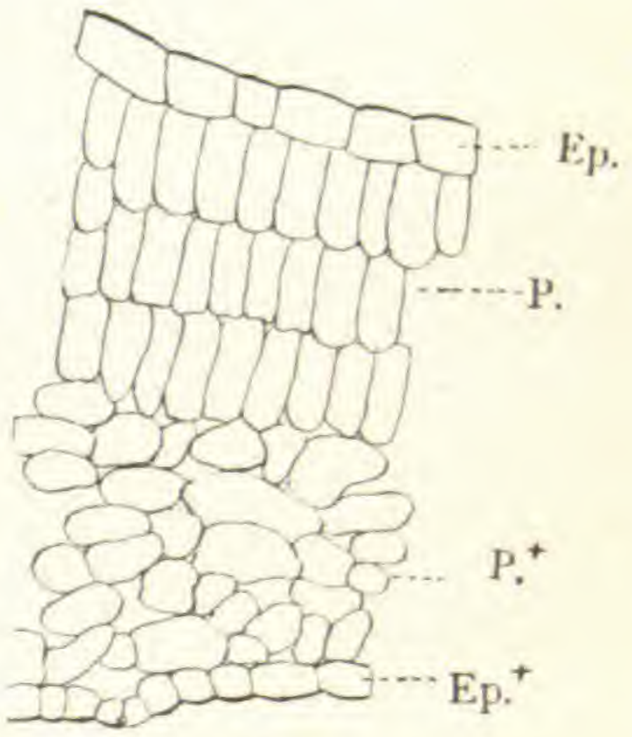

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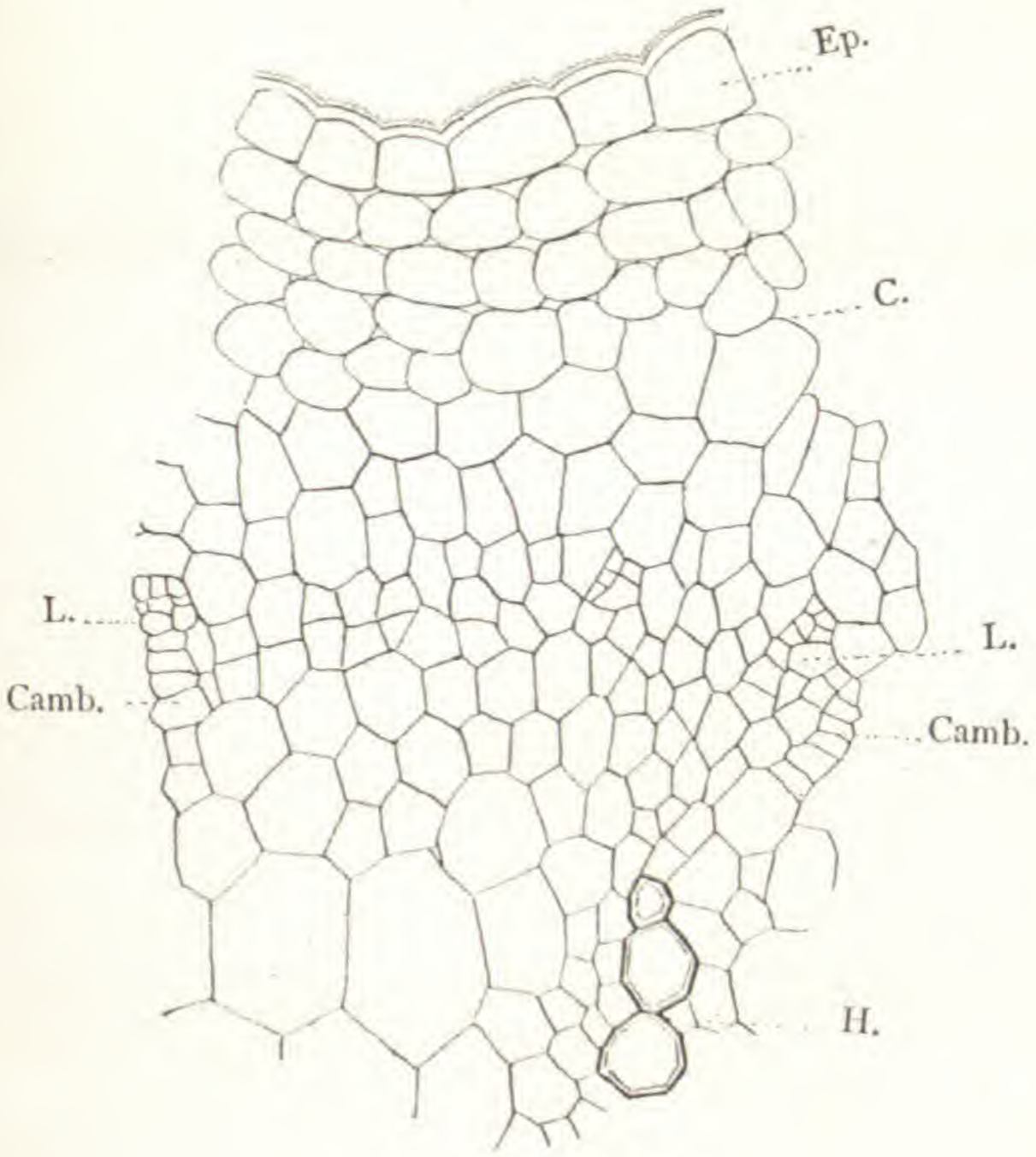

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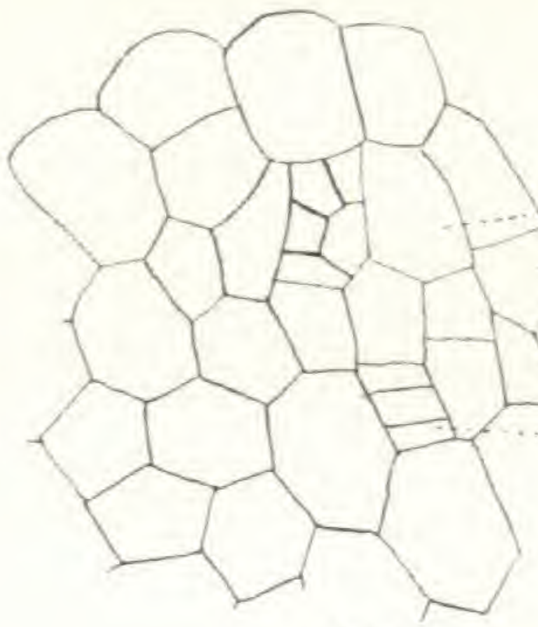

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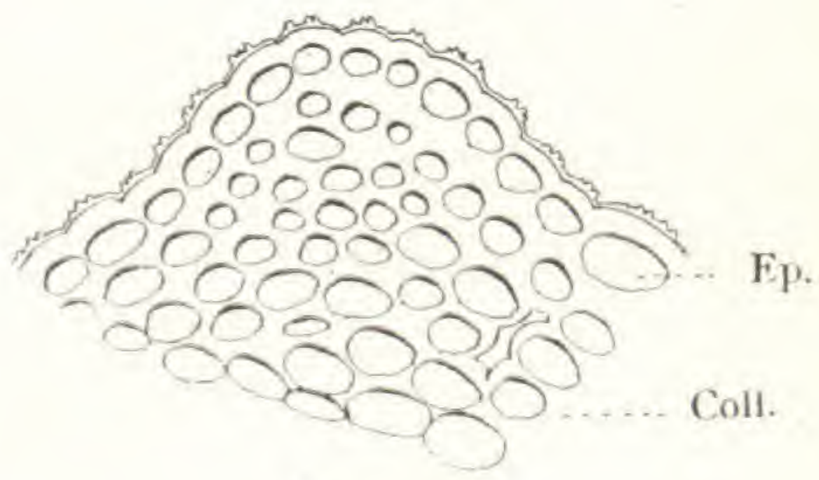

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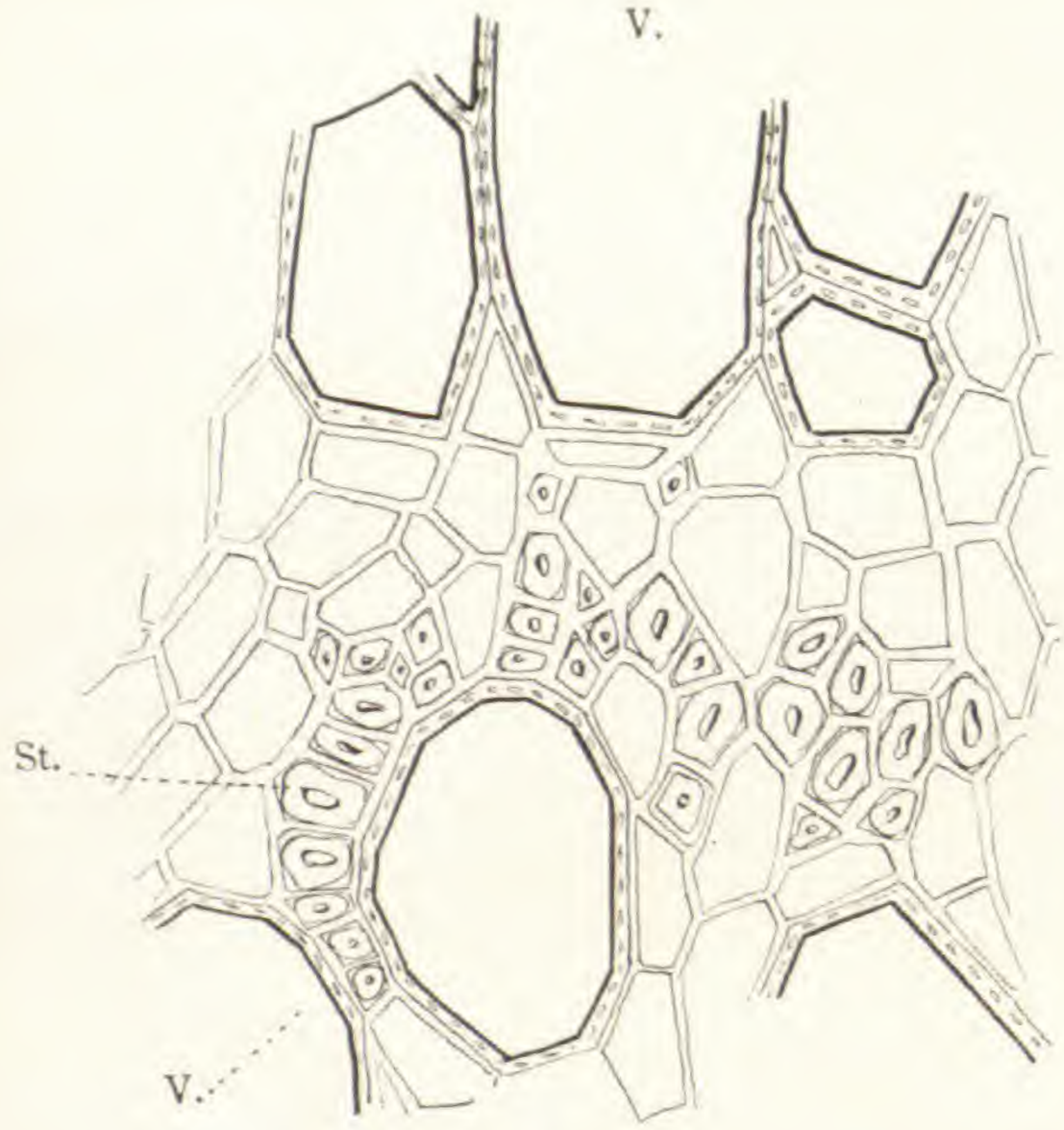

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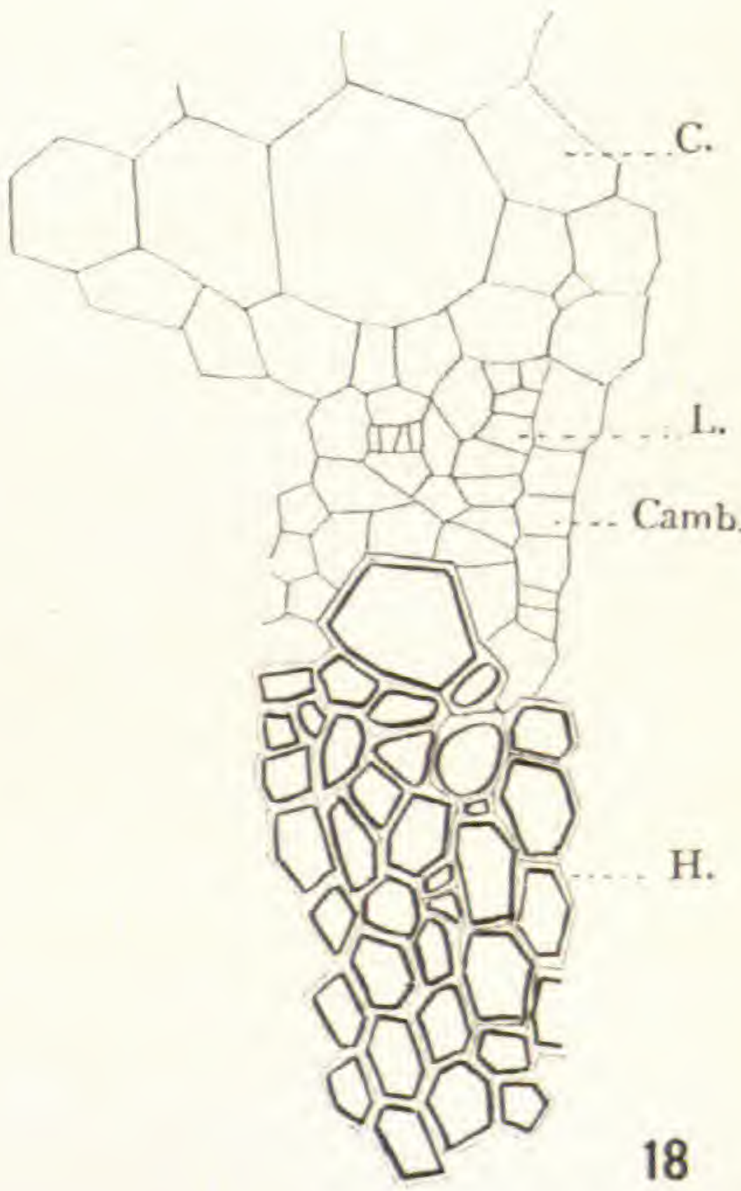

18

CORRELATION WORK IN PEAT-LAND PROBLEMS

A. P. DACHNOWSKI

A detailed account has recently been published by the writer showing for the first time the different types of plant organic material which are found in peat deposits within the glaciated area of this country.[1]

It was pointed out that this aspect of the peat problem had a practical importance for the close correlation which exists between the botanical composition of the different types of peat material and its corresponding physical, chemical, bacteriological, or other merits. Research of preliminary character along these lines on at least two distinct types of peat was reported in 1912.[2]

More extended field work, including peat in the southern states and the Pacific Coast, not only has confirmed that further research in these directions would be very desirable, but emphasized that it is precisely this information upon different types of peat material which supplies the essential criteria concerning the development and structure of peat deposits. Moreover, the facts are of fundamental importance also in the selection of workable tracts and in questions relating to the best means and practices of utilizing this source of national wealth for intensive or diversified agricultural and industrial purposes.

Peat deposits afford a large and profitable scope for a combination of scientifically directed industrialism and agriculture. They are an important national asset for fuel and power, for food and for certain products, and their best development will come when industries move to or near well-chosen, interconnected accumulations of peat, in order to insure a regular and continuous supply of different materials and to meet the demands of different markets. A great advantage would result from a better understanding of peat

[1] DACHNOWSKI, A. P., Quality and value of important types of peat material. U.S. Dept. Agric., Bull. 802, 1919.

[2] ———, Peat deposits of Ohio. Geol. Survey Ohio Bull. 16., pp. 424. *pls. 8, figs. 29.* In cooperation with the U.S. Bureau of Mines, 1912.

deposits and what they mean as raw materials to certain industries and to the investigators themselves.

Whatever the purpose for which a peat deposit may be used, it should clearly be understood that individual deposits of peat material present considerable variation, both in structure (the relative position of different layers) and in content (the character and quality of the different types of plant remains). All deposits are not the same; they will not yield to the same treatment, and they do not have the same value for scientific studies or for crops and for manufacturing purposes. It is largely on account of the failure to recognize these differences in stratification and in the quality of the peat materials that much of the scientific work in chemical analyses, in fuel and fertilizer determinations, in absorption and other data in the field of American peat investigations does not meet practical requirements. Inability to select suitable peat materials and workable deposits has made the peat-land problem difficult and uncertain. Crop yields and manufactured peat products have been unsatisfactory in many cases because definite information is not available as to the character of peat deposits in the United States, their actual acreage, and distribution. The data are still lacking on which to base the percentage of peat-land now in use and the real value of the unused areas of peat-land in this country.

This situation is significant in the efforts now being made to use these resources for fuel. It is no doubt possible, in most cases, to extend the agricultural uses of peat deposits and at the same time to meet the industrial needs with suitable deposits for centralized power stations or other manufacturing interests. At present these problems are not being met with the aid and cooperation necessary for various sections of the country. A comprehensive national program or policy of peat-land utilization may now be formed with safety. It should provide for the present and future needs of peat problems, for the conservation of unworkable deposits of peat, and for the best methods of combining agricultural and industrial interests where conditions favor the production of fuel and finished peat products as well as the demand for food. Furthermore, the basic principles and characteristics governing the utilization of peat deposits should be set forth for the

education of those concerned with the practical management of peat-lands. It is this phase of the problem concerning which the least has been done.

The basic importance of the stratigraphy of peat deposits and of development methods and principles in the interpretation of plant remains, such as peat deposits represent, has not as yet been generally recognized in this country. The investigator who views products and processes from the genetic and correlational standpoint has not become as prevalent in the field of peat investigations as one might surmise, and naturally there is still wanting the proper understanding of the profile structure of deposits which would make possible a satisfactory coordination of scientific activities as well as the effective agricultural or industrial use of specific peat-land areas. The scientific as well as the economic consequences of peat deposits under utilization are only made clear by a knowledge of the structure of the deposits. Not only commercial considerations but also the grasp of past and present modifying field conditions, the habit of regarding peat deposits from the broader scientific, even though at times rather theoretical, point of view will further and extend the possibilities of peat-lands.

To obtain information on yields and cost of production, or pertaining to experimental work which will permit interpretation of results, there must be more definite knowledge than is available now in regard to peat deposits and their materials. There is need, among other things, of a comparative study of the structural features of American and European peat deposits. The areas selected should comprise the latest and most authoritative investigations of workers in peat problems. The aim should be to state the stratigraphic facts fully as the data available permit, and it should include views, correlations, and, where the scope of the work warrants, advice on matters pertaining to investigations or securing information in the general field of peat-land problems.

The method of procedure in comparing profile features of peat deposits should be based on the botanical composition and physical appearance of the layers of peat. These furnish the information that strata of a certain type occur in certain localities on the American and European continents; that they replace one another, the

later ones being superimposed upon earlier layers; that they were formed in the course of a characteristic sequence or succession of vegetation units; and that the layers are more or less evidently connected with responses to changes in basic habitat factors.

It is of great importance to realize the influence which structural differences in the peat deposits exert upon the progress of peat-land agriculture, upon the advancements in the peat fertilizer industry, and upon improvements in mechanical devices for excavating, pulping, drying, or converting peat into fuel and other products.

Peat investigations in this country have reached the stage where basic correlations are possible between pleistocene geology, the distribution of peat deposits and their post-glacial vegetation units, and the climatic factors which in the past controlled the development and structure of peat accumulations. Clements[3] has recognized the need of extending peat investigations into the past, correlating geology, climatology, and the migration of former plant populations. A complete study of peat deposits is no longer possible without the aid of other sciences. It will now be practicable to extend European investigations dealing with climatic changes to the morainic systems of North America and to show whether or not glaciations have been contemporaneous, whether they depended upon general or local causes, and whether plant populations have immigrated and were affected by alternating dry and humid periods.

The information upon the different types of peat material offered in Bulletin 802 will aid in a tentative way, it is hoped, toward a solution of various peat-land problems. There are numerous questions in physical, chemical, and bacteriological studies, and also in physiological investigations dealing with peat materials which can now be attacked more successfully from this new standpoint.

The improvement of the present situation in matters of drainage, the management and the general uses of peat deposits for agriculture and for technical industries, should become obvious if consideration is given to the structural differences of deposits, especially to those deposits where systematic field experiments are to be carried out or various lines of practices are to be tested. Peat-

[3] CLEMENTS, F. E., Plant succession: An analysis of the development of vegetation, pp. 512. Carnegie Inst. Washington, publ. 242. 1916.

land differs very much from that of mineral soils. The bulk of a peat deposit is water and must contain not less than 65–70 per cent of water to be serviceable for the growth of crops. Moreover, in the great manufacturing states, particularly of the east and south, workable peat deposits are often involved which have a considerable acreage. On account of the fact that drainage and the utilization of such areas must frequently ignore state lines and control canals, dams, and the use of water for transportation or for irrigation purposes, the lines along which production should arise within certain selected areas of peat call not only for policies and organization, but also for extensive operations and for the collective working and association of small holdings into larger units.

It is not too late to adopt a national policy with regard to the conservation and utilization of peat deposits that are too large for individual enterprises, or which have been reserved for colonization. The only safe course is to determine carefully the character of the peat-land in the various regions of this country, and to lay a foundation for the methods and practices which will convert suitable land into productive areas. This policy of aiding and cooperating with the several interests should have national importance as well as state and local significance.

The relatively small quantity of plant remains in any peat deposit with a 65–70 per cent water content has not only unusual physical properties, but it contains various groups of organic compounds of great technical importance, and provides also the culture medium for bacterial organisms by means of which the organic matter may be made invaluable to agriculture. Peat materials are usually deficient in mineral salts suitable for the growth of cultivated crops, and they are not well balanced in fertilizer constituents. It is superfluous, therefore, to point out that the burning of organic material to increase the productiveness of the land is an erroneous practice which should be strongly condemned. As a general policy extensive drainage projects far in advance of the actual utilization of the peat deposits concerned should not be regarded as economical.

Engineers in various professions, after a careful study of peat-lands in relation to the nitrogen problem and the question of central electric power stations, have arrived at the conclusion that we are

only on the threshold of the great returns which workable peat deposits hold out to the industries combined with agricultural production. Until field work and experiments in this country begin to be conducted systematically on peat deposits, however, with a well-understood profile structure, and provision made for complete and continuous records, the methods and the results obtained in Germany, Sweden, and Holland will remain the chief sources of information and of practice. A critical and comparative study of the structural features and field conditions of American peat deposits is alone decisive. It is prerequisite in establishing the scientific foundation and the best practices which are necessary to successful utilization of peat-lands in this country.

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WASHINGTON, D.C.

ORIGIN OF MECHANISM OF HEREDITY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 274

MERLE C. COULTER

The gross features of the mechanism of heredity have become features of general knowledge. The majority of biologists think of heredity in terms of determiners located upon the chromosomes. There are certain critical details of the mechanism, however, which still remain profoundly obscure. Little is known of the exact nature of the determiners themselves. The orderliness in the behavior of the determiners, that is, how they are "released" to express themselves only at the appropriate moments in the life history of the organism, seems not to have been clearly visualized. Finally, the possible origin of this mechanism of heredity is seldom even discussed. The present paper suggests, although only in a very brief and general way, certain answers to these questions.

It seems safe to assume that the most primitive organism lacked not only an organized nucleus, but even the components of a nucleus. A consideration of the activities of such an organism will reveal a suggestion as to the origin of the hereditary mechanism, provided, of course, that the assumptions are sound. The metabolism of this primitive organism, in certain fundamental features, will be similar to that of all organisms. Raw materials will be taken in and transformed to provide building materials and energy. If the raw materials be pure and the machinery of the protoplast perfect, this transformation will be complete, so that all the raw materials taken in will be transformed and used. Actually, however, the raw materials provided are never quite pure, and the machinery of the protoplast, although infinitely more efficient than any man-made machine, must be subject to certain flaws and frictions of its own. The transformation and use of materials, therefore, will not be complete; certain waste materials and by-products will remain. We are not concerned with the waste material; it is the fate of the by-products which is significant.

Certain of the by-products may be insignificant in their influence upon the protoplast. Others will undoubtedly be toxic in their effect, as many investigations upon auto-intoxication have gone to show. Primitive excretory systems, developed primarily for the disposal of waste materials, may remove a considerable part of these by-products. Thorough cleansing of the protoplast by this method, however, is impossible. Inevitably by-products will accumulate with the age of the organism. In fact age itself, in other than the purely chronological sense, is probably accounted for by this very accumulation of by-products. The toxic influence of these by-products will interfere with the efficient working of the machinery of the protoplast, and metabolism will be slowed down; hence "old age." Rejuvenescence occurs with cell division, because at cell division the protoplast is cleansed of many of these toxic by-products. This cleansing probably involves both physical and chemical forces. Physical reorganization at cell division will explain the exposure of these by-products; chemical oxidation will account for their removal (as toxins).

Again, were the machinery of this cleansing process a perfect one, rejuvenescence would be complete. Actually, however, the cleansing of the protoplast at cell division is not (or is not always) absolutely thorough. A few of the by-products pass over to the daughter protoplasts. The daughters, therefore, start life with a few by-products which the mother did not possess at the beginning of her life. Since these by-products are toxic and impair or retard metabolism, it is evident that the daughters are, at birth, slightly "older" than was the mother.

A series of repetitions of this performance through successive generations will have a cumulative effect. As a consequence, not only does the individual grow old through ontogeny, but, in a very real sense, the whole race is gradually aging through phylogeny. Evidence is not lacking that the higher organisms, cell for cell, have a lower rate of metabolism than do the more primitive ones. This is a statement of the quantitative effect of these by-products. It is their qualitative effect, however, that casts light upon the origin of the hereditary mechanism.

The by-products which originally accumulated in the protoplast were of various types. Some were very toxic, and these, if they were not immediately eliminated, resulted in the death of the organism. Others were less toxic, relatively more harmonious with the protoplast itself. These last, since they were not immediately fatal, stimulated an adaptive response on the part of the protoplast.

As to the general nature of this adaptive response, an important assumption must be made. Recent researches upon mammals have revealed in these organisms the power to develop antitoxins. The presence of a small quantity of toxin stimulates the organism to an adaptive response, the development of an antitoxin specific for the toxin present. This power is probably one of the fundamental characteristics of all protoplasm, being present even in the most primitive organisms.

Certain by-products in the primitive organism, only slightly toxic in their effect, stimulated it to produce an antibody. The protoplast is doubtless a colloidal system, and we may consider antibodies in the following light. The antibody counteracted the influence of the toxic by-product by insulating it from contact with the protoplast. Antibodies were probably developed most successfully for those by-products which were the least toxic in their effect. These by-products then became insulated by the antibodies. This insulation was significant not only in cutting off the influence of the by-product upon the protoplast, but in another respect also. At cell division this by-product, even though exposed, is not oxidized because of the protection afforded by the antibody which insulates it. It is probably this mechanism, for the most part, which accounts for the fact that some of the by-products are passed on to the daughter protoplasts, as mentioned before. These by-products are the primitive bearers of hereditary characters. The program carried out by the primitive hereditary mechanism is as follows.

The life of the primitive organism, like that of all organisms, involves a series of reactions. Early in the life of the organism there is present a certain reaction system, characterized by certain physical and chemical conditions. For a time the reaction system

as a whole maintains a sort of equilibrium, in which only reactions of a certain type are possible. This may be referred to as the *A* equilibrium. Inevitably, through the accumulation of certain materials, the *A* equilibrium will be upset. After a period of readjustment, the *B* equilibrium will succeed; this will be followed by the *C* equilibrium, and so on. The total number of distinct equilibria in the life program of any organism probably is in rough proportion to phylogenetic age.

Taking as an example the *X* equilibrium, certain questions may be considered. What is it that accounts for the existence of the *X* equilibrium? It is the inevitable result of reactions which took place during the *W* equilibrium. The *W* equilibrium may be similarly accounted for by the previous existence of the *V* equilibrium. The program is an inevitable one, and will be followed during each generation.

Under conditions imposed by the *X* equilibrium, only reactions of a certain type are possible, and these may be referred to as the *x* reactions. A number of *x* reactions are possible, x_1, x_2, x_3, etc. Chance conditions (environment, directly or indirectly) will determine which of these will take place. Whichever takes place, there will result a by-product, and this by-product will be of the *x* type. Even more specific than this, the x_3 reaction will result in and be characterized by the x_3 by-product. As the existence of the *X* equilibrium was inevitable, there will inevitably be laid down one of the *x* type of by-products. Since, however, it was chance which specifically selected the x_3 reaction, this same chance is indirectly responsible for the by-product x_3, rather than x_1, x_2, or any of the other possibilities.

The by-product x_3 will exist in an active state and exert some influence upon the protoplast so long as the *X* equilibrium continues. The eventual disappearance of the *X* equilibrium will be accompanied and characterized by the insulation of by-product x_3 by means of a specific antibody which has been developed by the protoplast. The *Y* equilibrium will follow; and, just as the *X* equilibrium was characterized by the free active existence of by-product x_3, one of the characteristics of the *Y* equilibrium will be the existence of x_3 in an insulated inactive condition. The by-

product x_3, insulated by its antibody, will pass on at cell division to the daughter protoplast. Early in the life of the daughter there must exist an A equilibrium. The inevitable program will then be followed, until finally the X equilibrium is reached. A very critical assumption is made at this point. The insulation of x_3 by its antibody is a phenomenon of colloidal chemistry. Similar colloidal reactions are known to be reversible. The formation of the antibody for x_3 took place at the inception of the Y equilibrium, which was characterized by the effective insulation of x_3. The X equilibrium, however, which now recurs during the following generation, is conducive to the free and active existence of any by-product of the x type. When the X equilibrium is reached in the life of the daughter protoplast, therefore, a dissolution of the antibody will occur and x_3 will be released.

With the X equilibrium now in existence, it is certain that reactions of the x type will take place. Which one of the possible x reactions occurred was in the first generation a matter of chance. In the present instance, however, the presence of by-product x_3 will exert a determining influence. The result, eliminating external stimuli of an unusual intensity, will be that the x_3 reaction and the x_3 by-product again stimulate the protoplast in a characteristic manner, developing in the daughter the same characteristic that was present at a similar stage in the life of the mother.

This theory accounts for the origin of the hereditary mechanism in terms of by-products and antibodies which insulate them. These various antibodies must form an important constituent of "modern" chromosomes, but there must also be present some more stable and homogeneous framework.

The release of the determiners (by-products) at the appropriate moment is referred to phenomena of colloidal chemistry. It is an open question whether this release is reflected by visible changes in the chromosomes. If so, a given locus on a chromosome should be seen in a loose or "open" condition only during a brief phase of the life history. No doubt this point would be hopeless to ascertain in any very accurate way.

As for the determiners themselves, these are visualized as by-products of metabolism, chemically active substances. The

reaction system (for example, X equilibrium), which arises as the result of an inevitable sequence of events, determines what general type of reaction shall take place at a given phase in the life history. The by-product (for example, x_3) merely decides which of a number of possible reactions within this general type shall be the one chosen.

The origin of a given by-product was accounted for by chance environmental conditions. The environment referred to may well have been the external environment in the case of the simpler organisms, but must be the internal environment in the more complex. This seems also to provide sufficient basis to explain the small degree of inheritance of acquired characters that has been said to take place.

UNIVERSITY OF CHICAGO

BRIEFER ARTICLES

EFFECT OF ZINC AND IRON COMPARED WITH THAT OF URANIUM AND COBALT ON GROWTH OF ASPERGILLUS

It has been shown in a preceding publication[1] that treatment of Pfeffer's nutrient solution with calcium carbonate at an elevated temperature removes from the solution the last traces of iron, zinc, and probably the other heavy metals to a high degree. It has also been shown that Pfeffer's solution so treated supports but a minimal growth of *Aspergillus niger*, but that the addition of both iron and zinc results in a phenomenal increase in growth as measured by the dry weight formed. The evidence presented, while showing that the presence of zinc in the cultural solution is just as necessary for *A. niger* as the presence of iron, did not include the action of other heavy metal salts.

Theoretically the extension of these observations to include other heavy metal salts is of importance, inasmuch as some light would be thrown on the parts played by iron and zinc salts in the metabolism of this fungus. If other heavy metal salts can replace either or both iron and zinc salts, as is commonly stated, then iron and zinc are not essential for the growth of *A. niger* and play the part of "chemical stimulants." If they cannot be replaced by other heavy metal salts, then they possess properties that remove them from this category and place them among the elements essential for the growth of *A. niger*.

For the purpose of this comparison only $Co(NO_3)_2+6H_2O$ and $UO_2(NO_3)UO_2(NO_3)_2+6H_2O$, both Kahlbaum reagents, were available, and these were therefore used. The method used has already been described (see footnote 1).

The results with $Fe_2(PO_4)_2+4H_2O$ and with $ZnSO_4+7H_2O$ apparently point to an incomplete action of the treatment with calcium carbonate, especially in reference to the removal of iron, since the cultures to which only zinc was added gave dry weights much higher than usual (see footnote 1). In the series containing both iron and zinc the usual phenomenal increases in dry weight occurred. Also regarding acidity it will be noticed, what has already been pointed out, that a parallelism seems to

[1] STEINBERG, R. A., A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. Amer. Jour. Bot. **6**:330. 1919.

TABLE I

EFFECT ON GROWTH OF *A. niger* OF ADDITION OF IRON, ZINC, COBALT, AND URANIUM SALT TO TREATED PFEFFER SOLUTION (STRAIN W, 12)*

SOLUTION	YIELD	PH	SPORULATION
	$FePO_4$		
$FePO_4$/flask	(gm.)		
0 mg	0.049	3–4	Fair
1	0.044	3–4	Fair
5	0.046	3–4	Fair
20	0.047	3–4	Fair
50	0.072	3–4	Fair
	$ZnSO_4+7H_2O$		
Zn/liter			
0 mg	0.026	3–4	Fair
1	0.060	3–4	Fair
5	0.061	3–4	Fair
20	(0.267)	2–3	Good
50	(0.362)	2–3	Practically sterile
	1.0 mg. $FePO_4$/flask + zinc†		
Zn/liter			
0.1 mg	0.377	2–3	Good
0.5	0.771	1–2	Fair
1	0.694	1–2	Fair
10	0.688	1–2	Fair
20	0.723	1–2	Fair
50	0.759	1–2	Fair
	1.0 mg. $FePO_4$/flask + $Co(NO_3)_2$		
$Co(NO_3)_2$/flask			
0.1 mg	0.072	3–4	Practically sterile
0.5	0.058	3–4	Practically sterile
1	0.053	3–4	Practically sterile
10	0.049	3–4	Practically sterile
20	0.033	3–4	Practically sterile
50	0.026	3–4	Practically sterile
	1.0 mg. $FePO_4$/flask + $UO_2(NO_3)_2$		
$UO_2(NO_3)_2$/flask			
0.1 mg	0.048	3–4	Practically sterile
1	0.062	3–4	Practically sterile
5	0.062	3–4	Practically sterile
10	0.058	3–4	Practically sterile
20	0.069	3–4	Practically sterile
50	0.080	3–4	Practically sterile

*The compounds used in the studies were Baker's "analyzed" KH_2PO_4; $ZnSO_4+7H_2O$; and $CaCO_3$; and Merck's "reageant" NH_4NO_3; $MgSO_4+7H_2O$ and "highest purity" sucrose. All other compounds were the Kahlbaum.

† The zinc ($ZnSO_4+7H_2O$) was added to these cultures 3.5 days after inoculation.

TABLE I—*Continued*

Solution	Yield	Ph	Sporulation
	0.1 mg. Zn/liter+$Co(NO_3)_2$		
$Co(NO_3)_2$/flask	(gm.)		
0.1 mg	0.075	3–4	Practically sterile
0.5	0.103	3–4	Fair
1	0.093	3–4	Fair
10	0.190	3–4	Excellent
20	0.180	3–4	Excellent
50	0.192	3–4	Excellent
	0.1 mg. Zn/liter+$UO_2(NO_3)_2$		
$UO_2(NO_3)_2$/flask			
0.1 mg	0.062	3–4	Practically sterile
1	0.053	3–4	Practically sterile
5	0.053	3–4	Practically sterile
10	0.138	3–4	Fair
20	(0.462)	2–3	Fair
50	0.095	3–4	Practically sterile

exist between increase in dry weight, increased acidity, and decrease in sporulation.

Examination of the data obtained with $Co(NO_3)_2+6H_2O$ and with $UO_2(NO_3)_2+6H_2O$ shows the surprising result that the phenomenal increases in dry weight ascribed to these metals do not occur.[2] The increases in dry weight, excepting for a few cases (zinc plus cobalt salts), are within the experimental error of the observations. The unincreased acidity of the cultures supports the viewpoint that no increases in growth had been obtained. As concerns sporulation, there seems to be no regularity in the cultures containing cobalt or uranium, and while it is interesting to note that in the last series, containing only cobalt and uranium, sporulation took place, it would be an error to suppose that this necessarily implies that neither zinc nor iron is needed by *A. niger* for spore formation. The probable explanation is the presence of traces of iron and of zinc in the cobalt and uranium salts.

The lack of any marked increases in dry weight brought about by the addition of cobalt and uranium salts to the cultural solution is entirely contrary to the results already recorded in the literature. The explanation apparently best fitting the facts is that partial replacement

[2] Le Pierre, C., Remplacement du zinc par l'uranium dans la culture de l'*Aspergillus niger*. Compt. Rend. **156**:156. 1179. 1913; Richards, H. M., Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. Jahrb. Wiss. Bot. **30**:665. 1897.

may occur and does occur only when iron and zinc are not present in too low amounts. It would seem certain, considering the many described cases of increased growth caused by heavy metal salts, that the contamination of the "stimulants" with iron and zinc could not have occurred without some exceptions if contamination of the "stimulants" were the cause.

It is not impossible that the action of iron and of zinc and the action of the other so-called "chemical stimulants" will be found to be intimately connected with the increased acidity of the nutrient solution. This hypothesis has already been discussed in a former paper. The assumption of a direct action of the "chemical stimulants" must be dropped, however, since these experiments make apparent that their effects are in some manner related to those of iron and zinc, which latter are directly correlated with acid production by the organism. This explanation can also be extended to include the alkaloids, since the extremely high concentrations in which they are effective in bringing about increased growth would eliminate from consideration their action as alkaloids, and their chemical properties as free base and as acid salts are well known. The action of the chemically inert "stimulants," such as ether, chloroform, etc., may in the same manner be laid to the disturbance of the respiration processes leading to an accumulation of organic acids. The fact that free oxalic acid, in common with other acids, can bring about this increased growth, etc., renders this supposition not improbable, in spite of the fact that it is commonly stated that the products of catabolism of an organism exercise a harmful effect on its growth.[3] Clearly we have here a case in which a product of the metabolism causes an acceleration of the rate of growth of the organism.

These studies must be looked upon, therefore, as a confirmation of the conclusions arrived at by RAULIN (Études chimiques sur la végétation. Ann. Sci. Nat. Bot. V. **11**: 93. 1869) and by JAVILLIER (La Présence et la rôle du zinc chez la plantes. Theses. Paris. 1908) that both iron and zinc are essential for the growth of *Aspergillus niger*, and the assumption must be made that when iron and zinc are present in favorable amounts a partial replacement of either or both these elements can occur by the so-called "chemical stimulants."—R. A. STEINBERG, *Bureau of Plant Industry, Washington, D.C.*

[3] PFEFFER, W., Physiology of Plants. I. Oxford. 1897.

CURRENT LITERATURE

NOTES FOR STUDENTS

Phragmospheres and binucleate cells.—BEER and ARBER[1] maintain that there is a binucleate phase during the development of the parenchymatous tissues of the higher plants, which is preceded, and usually succeeded, by a uninucleate phase. They conclude that the binucleate condition is invariably brought about by mitosis, and state:

> The division occurs normally in the earlier stages, up to the period at which the two daughter nuclei are at the poles of the spindle, while the cell-plate is just being initiated. But at this point the mechanism seems to break down and the cell-plate is resorbed, while the phragmoplast, with its associated cytoplasm, goes through a singular metamorphosis. It becomes vacuolate in the center and develops into a hollow sphere which gradually grows until it incloses both the daughter nuclei, and then, by its further extension, ultimately merges into the cytoplasm lining the cell wall. For this hollow shell we have proposed the term "phragmosphere." In some cases it is exceedingly well defined and stains deeply, giving the sections in which it occurs a curious appearance of exhibiting cells within cells.

The writer[2] recently has described certain cytological phenomena which appear to be significant in this connection. In longitudinally dividing cells of the cambium of the higher plants the central spindle expands laterally by the addition of peripheral fibers, and gradually assumes the form of a disk. The connecting fibers and later the accessory fibers thicken to produce a cell plate and then disappear, leaving a circular rim of kinoplasm. In tangential longitudinal sections of the cambium, this ring-shaped aggregation of kinoplasmic fibrillae, phragmoplast, forms a halo about the daughter nuclei and gives the impression of a "cell within a cell." It increases in circumference, by the addition of new peripheral fibers, until it intersects the radial facets of

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[1] BEER, R., and ARBER, AGNES, On the occurrence of binucleate and multinucleate cells in growing tissues. Ann. Botany **29**:597–598. 1915.

———, On the occurrence of multinucleate cells in vegetative tissues. Proc. Roy. Soc. B. **91**:1–17. 1919.

ARBER, AGNES, Studies on the binucleate phase in the plant cell. Jour. Roy. Micr. Soc. March 1920. 1–21.

[2] BAILEY, I. W., Phenomena of cell division in the cambium of arborescen gymnosperms and their cytological significance. Proc. Nat. Acad. Sci. **5**:283–285 1919.

———, The formation of the cell plate in the cambium of the higher plants. Proc. Nat. Acad. Sci. **6**:197–200. 1920.

———, The significance of the cambium in the study of certain physiologica l problems. Jour. Gen. Physiol. **2**:519–533. 1920.

the protoplast. At this stage in its development the kinoplasm disappears from two sides of the phragmoplast, leaving two entirely separate aggregations of kinoplasmic fibrillae. These rod-shaped masses of kinoplasm (kinoplasmasomes) move in opposite directions, thereby extending the cell plate, until it eventually reaches the two ends of the protoplast. This type of cytokinesis is not confined to the cambium, but occurs in other somatic tissues, in elongated or much flattened cells whose planes of division have one long and one short dimension.

A comparative study of cytokinesis in different somatic tissues and in cells of different shapes and sizes indicates that the various types of cell plate formation, described by TREUB, STRASBURGER, SCHÜRHOFF, and the writer, are but different phases or stages of a single fundamental type of cytokinesis. The particular expressions of the phenomenon which may occur in a given cell are dependent upon the dimensions of the latter, its plane of division, and the size and location of the nucleus. Thus, in very small isodiametric cells, having a large centrally located nucleus, the cell plate quickly intersects the walls of the cell, without any extensive lateral growth of the phragmoplast. In larger elements there is sufficient room for the phragmoplast to attain the "halo" stage before the cell plate intersects the sides of the protoplast.

The striking similarity between BEER and ARBER'S figures of sections of "phragmospheres" and polar views of normal ring-shaped phragmoplasts, such as occur in the cambium and other tissues of the higher plants, raises the question whether the phenomena encountered by these investigators are not actually stages in the division of parenchymatous cells. Although they state that the whole phragmoplast with its associated cytoplasm becomes transformed into a hollow sphere which incloses the daughter nuclei, and that the cell plate is resorbed without forming a membrane, they present no conclusive evidence in favor of such a hypothesis. For example, they give no critical figures or detailed descriptions to elucidate various stages in the formation of the phragmospheres or the resorption of the cell plate. They state that the phenomena occur in cells which are not forming division membranes, yet the binucleate phase is admitted to reach its most characteristic expression in young active tissue, just previous to the maximum period of growth undergone by the region of the stem, leaf, or root in which it occurs. Of course, the fact that individual cells are increasing in diameter does not indicate necessarily that they have ceased to divide in a given plane. Although the material that I have studied is extremely favorable, owing to the large size of the cells, nuclei, and division figures and their symmetrical arrangement, I have frequently found it difficult to determine, in a given plane of section, whether cells contained more than one nucleus each. This is due to the fact that nuclei which appear to lie within a single protoplast, that is in the same focal plane, subsequently are found to be separated by thin, recently formed membranes or cell plates. BEER and ARBER'S conclusions seem to have been drawn largely from the study of transverse sections of stems and roots.

In view of the important bearing of BEER and ARBER'S conclusions upon various cytological and physiological problems, it is to be hoped that future investigations may throw some light upon the following questions:

1. Are BEER and ARBER'S phragmospheres actually hollow spheres of kinoplasm or ring-shaped phragmoplasts such as occur in other tissues of the higher plants?

2. If they are ring-shaped phragmoplasts, as seems probable, do they form cell plates?

3. Are the nuclei of the "binucleate cells" separated by a thin membrane, or does the cell plate disappear without forming a membrane?

4. Do the nuclei of multinucleate cells arise by the same type of nuclear division as the nuclei of binucleate cells?—I. W. BAILEY.

Hepaticae.—Among recent publications on the Hepaticae by EVANS are the following. In continuation of studies of the New England Hepaticae,[3] three species of *Nardia* are fully discussed, one of them being described as new. In continuation of the North American Hepaticae,[4] other species of *Nardia* are considered, also additions to the flora of the United States, extensions of range, and clearing up some difficulties in nomenclature. A taxonomic study of *Dumortiera*[5] contains a full discussion of the two species as to structure, classification, stations, and literature.

A new *Riccia*[6] (*R. bistriata*) from Peru presents a noteworthy feature in "the peculiar bands of thickening which are found in the walls of the green cells," a feature which has not before been noted in the Marchantiales. Three species of *Asterella*[7] from South America are presented as new combinations, transferred from *Fimbriaria*. They are not known to extend into North America; in fact, of the 15 North American species, only two are known to extend into South America.—J. M. C.

Ripening of tomatoes.—SANDO[8] finds that the maturity of a tomato is dependent upon its age and not upon its size in the growing conditions under which he worked. His analyses show that throughout the ripening period there is an increase in moisture, acids, and sugars, and a decrease in solids, total nitrogen, starch, pentosans, crude fiber, and ash. Sugars increase from

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[3] EVANS, A. W., Notes on New England Hepaticae. XV. Rhodora **21**:149–169. *pl. 126. figs. 14.* 1919.

[4] ———, Notes on North American Hepaticae. VIII. Bryologist **22**:54–73. *pl. 2. figs. 15.* 1919.

[5] ———, A taxonomic study of *Dumortiera*. Bull. Torr. Bot. Club **46**:167–182. 1919.

[6] ———, A new *Riccia* from Peru. Torreya **19**:85–88. *fig.* 1. 1919.

[7] ———, Three South American species of *Asterella*. Bull. Torr. Bot. Club **46**:469–480. 1919.

[8] SANDO, CHAS. E., The process of ripening in the tomato, considered especially from the commercial standpoint. U.S. Dept. Agric., Bull. 859. 1920.

25.66 per cent in the fruit 14 days old to 48.32 per cent in ripe fruit. Starch decreases in the same period from 15.84 per cent to 2.65 per cent. Fruit picked after it had started to color and allowed to ripen in free access of air showed practically the same composition and edible qualities as fruit ripened on the vine. Commercially ripened tomatoes, due to lack of free access of air, show very different composition and inferior taste. Lack of ventilation during ripening increased the acid content 138 per cent and decreased the soluble carbohydrate content 21 per cent. Commercially ripened tomatoes wrapped with one paper showed similar but less marked changes. SANDO is unable to explain why wrapping with three papers was less detrimental than wrapping with one.—WM. CROCKER.

Seed sterilization.—BRAUN[9] has developed what he calls the "presoak" method of seed sterilization. "The use of formalin and copper sulphate as now practiced usually causes retardation and injury to seed germination." He finds that he can eliminate this injury by soaking the wheat seeds ten minutes and covering them for six hours to allow them to absorb the adhering water. Then follows the usual treatment with the disinfectant. The presoak method often shows a noticeable stimulative effect. BRAUN speaks of presoaking as saturating the walls and cells of the seeds so as to dilute the disinfectants beyond an injurious concentration. He also speaks of presoaking as increasing the efficiency of the disinfectants by bringing the dormant bacteria and fungi into a vegetative state so they are more easily killed by the disinfectants. —WM. CROCKER.

Plant distribution in South Africa.—An analysis of the plant population of South Africa by BEWS[10] shows that while WILLIS's "age and area" theory may be in general accepted, its application is not feasible in the region under discussion, on account of the very great climatic variations. Species widely distributed in South Africa are found to belong to pioneer stages of the various plant successions, although not all pioneer forms are widely distributed. Many species with a restricted distribution are shown to belong to climax associations, especially in the coast belt forest of Natal.—GEO. D. FULLER.

Studies in Taraxacum.—STORK[11] has extended our knowledge of this interesting genus, discovering "parthenogenesis" in additional species, in the sense that the unfertilized egg develops an embryo, but it is a diploid egg. He also records some interesting variations in the genus, and gives an account of megasporogenesis in *T. erythrospermum*.—J. M. C.

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[9] BRAUN, HARRY, Presoak method of seed treatment: A means of preventing seed injury due to chemical disinfectants and of increasing germicidal efficiency. Jour. Agric. Res. **19**:363–392. 1920.

[10] BEWS, J. W., Plant succession and plant distribution in South Africa. Ann. Botany **34**:287–297. 1920.

[11] STORK, HARVEY E., Studies in the genus *Taraxacum*. Bull. Torr. Bot. Club **47**:199–210. 1920.

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